# Understanding metabolic phenomena accompanying high levels of yeast in broiler chicken diets and resulting carcass weight and meat quality changes

Vladana Grabež, \*,1,2 Bjørg Egelandsdal, \*,1 Ana Cruz, † Elin Hallenstvedt, † Liv Torunn Mydland, § Ole Alvseike, † Karoline Kåsin, \* Lene Ruud, \* Victoria Karlsen, \* and Margareth Øverland • §

**ABSTRACT** The use of yeast as a protein source was investigated in broiler chicken diets on carcass quality, storage stability, and metabolite changes in leg meat. Male Ross 308 chickens (n=100) were fed with one of 5 diets: control, control added 0.6% formic acid, or 3 diets where soybean meal was substituted with 10, 20, and 30% crude protein from inactivated yeast *Cyberlindnera jadinii* (CJ10, CJ20, CJ30, respectively). The yeast-containing diets reduced carcass weight, linoleic acid, and

warm-over flavor in chicken leg meat. Protein degradation-related metabolite biomarkers were upregulated in the leg of chickens that were fed yeast-containing diets, indicating an adaptive response to the loss of appetite. Chill-stored leg meat of birds fed yeast diets showed increased browning and metallic taste compared with those fed the control diet. The use of formic acid in the diet reduced cooking loss and had a positive effect on vitamin B content.

Key words: yeast, broiler chicken, leg meat, oxidative stability, metabolite changes

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#### INTRODUCTION

Yeast grown on lignocellulosic biomass as a source of second-generation sugars has been suggested as a sustainable local protein-rich feed ingredient for chicken meat production in countries with limited arable land. Cyberlindnera jadinii yeast scan be produced from various lowwaste agriculture and forestry (Bekatoru et al., 2006; Øverland et al., 2017). In addition, this yeast strain has a "Generally Recognized As Safe" (GRAS) status assigned by the Food and Drug Administration (FDA) and is used as a safe ingredient in food. C. jadinii is a high-quality protein source with a favorable amino acid composition and has shown to support high growth performance in diets for piglets (Cruz et al., 2019) and Atlantic salmon (Overland et al., 2013). Feeding diets with increasing levels of C. jadinii yeast to broiler chickens resulted in similar growth performance as the control when the yeast replaced 10% crude protein

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(CP) from soybean meal, but at higher inclusion levels (20% and 30% CP replacement), feed intake and weight gain of the birds decreased linearly (Cruz et al., 2020). Replacement of 20% and 30% soybean meal with *C. jadinii* grown on distillery vinasse in the chicken diet increased feed conversion (Rodríguez et al., 2013). However, weight gain and live weight of broiler chicken were not affected when 20% soybean meal was replaced with the yeast (Rodríguez et al., 2013). Thus, the substitution of soy protein up to 20% with brewer's yeast mixtures in grower diet showed no effect on the growth performance of chickens (Carías and Millán, 1996).

The addition of organic acids such as formic acid to diets can improve growth performance of broiler chicken (Khodambashi Emami et al., 2013). The inclusion of 1% formic acid in diets is regarded as safe (Ricke et al., 2020) but its effect in chicken feed may vary. At 0.5% inclusion level formic acid showed a positive effect on feed conversion ratio and live weight of broiler chicken (Ndelekwute et al., 2015; Ragaa and Korany, 2016). However, the addition of 0.5% and 1% of formic acid had no effect on growth Hernández et al. (2006) and live weight Sugiharto et al. (2019) of broiler chickens.

Growth performance, feed intake, and feed conversion ratio are commonly the focus when conventional protein sources are replaced with yeast (Øverland and Skrede, 2017; Agboola et al., 2021). However, limited

<sup>\*</sup>Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, NO-1432, Ås, Norway; †Felleskjøpet Fôrutvikling AS, NO-7018, Trondheim, Norway; †Nortura SA, NO-0513, Oslo, Norway; Department of Animal and Aquacultural Sciences, Faculty of Bioscience, Norwegian University of Life Sciences, NO-1432, Ås, Norway; and #Animalia, Økern, NO-0513, Oslo, Norway

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<sup>&</sup>lt;sup>1</sup>Authors contributed equally to this paper.

 $<sup>^2</sup> Corresponding \ author: vladana.grabez@nmbu.no$ 

knowledge exists of the effect of yeast and formic acid in broiler chicken diet on meat quality characteristics. Carcass weight and yield of birds were not affected by increasing level of C. jadinii yeast (at 10%, 20%, and 30% replacement of soybeanmeal) in broiler chicken diet (Rodríguez et al., 2013). Low inclusion level of Saccharomyces cerevisiae (1.5 and 2 g/kg) in broiler chick diets increased dressing percentage and weight of the chicken breast muscle (Ahiwe et al., 2020). Others have reported improved meat tenderness and oxidative stability when 3% of soybean meal was replaced with S. cerevisiae (Zang et al., 2005a,b), while replacement of 40% and 50% of soybean protein in with S. cerevisiae had positive effect on flavor properties of breast and leg muscles of broiler chickens (Janocha et al., 2011). Addition of 1\% formic acid to diet showed no effect on cut yields of chickens (Garciá et al., 2007; Sugiharto et al., 2019). The organic acid increased pH and lightness of chicken breast muscle, thus, reduced drip-loss (Sugiharto et al., 2019).

Protein synthesis in the skeletal muscle of fast-growing broiler chickens can be affected by feed intake and variations in dietary amino acids. Thus, protein metabolism in broiler chicken is inevitably linked with energy metabolism reflecting the complexity of possible metabolite changes in the muscle cell-induced with the replacement of soy with yeast protein. Therefore, metabolite changes in chicken leg meat should be addressed.

The aim of this study was, therefore, to investigate changes in carcass and leg meat quality of chicken described by Cruz et al. (2020) regarding inclusion level of *C. jadinii* yeast to broiler diet.

Our investigation describes the storage stability and metabolites of meat from broiler chicken fed diets added 0.6% formic acid or increasing levels of yeast compared with the meat from chicken fed conventional wheat—oat—SBM diets.

#### **MATERIALS AND METHODS**

## Management and Dietary Treatment

The present study used leg meat from the birds fed the growth performance experiment Cruz et al. (2020), as a muscle with more complex metabolic activity and possibly higher sensitivity to dietary changes. Briefly, a total of 1,250 male Ross 308 broiler chickens from a commercial hatchery were hosted at the experimental farm at the Norwegian University of Life Sciences. A total of 250 birds with an average initial body weight of 42 g  $\pm$  0.75 g per dietary treatment were housed in 5 pens (50 birds/pen) and fed 5 experimental diets with increasing levels of C. jadinii yeast for the starter period (day 0-9) and grower period (day 10-30) (Table 1). Table 1 shows the ingredient composition of the experimental diets.

The birds were handled according to local welfare laws and regulations, the Animal Welfare Act of December 28, 2009, and the local legislation derived from the directive 2010/63 EU of the European Parliament and

Council of September 22, 2010, on the protection of animals used for scientific purposes.

## Sample Collection

The study of carcass, thus, leg meat quality and metabolomic changes included 20 birds per dietary treatment (4 birds per pen randomly selected) at 30 d of age. The birds were transported to the commercial slaughterhouse (Nortura SA, Elverum, Norway) and processed while hanging on the slaughter line, scalded (57.5°C, 3 min, 9,500 animals/h), manually defeathered, and eviscerated. After evisceration, the carcasses were transferred to the chiller-tunnel at 2 to 4°C for 55 min and later in the chiller-room for 24 h until sampling. Cold carcasses were weighed and both legs (thigh and drum) were dissected at the hip and knee joints. Deboned and skinless leg meat was weighed and transported in plastic bags on ice from the slaughterhouse to the meat laboratory at the Norwegian University of Life Sciences in Ås, where all analyses were performed.

On the same day, one-half leg meat (thigh and drum) per bird was homogenized, vacuum packed, and stored at  $-80^{\circ}$ C until analysis. The remaining one-and-a-half leg from the same carcass was packed in a modified atmosphere. Each meat sample was placed in the amorphous polyethylene terephthalate tray (dimensions  $23.9 \times 12.5 \times 3.5$  cm) and sealed with ethylene vinyl alcohol top film using the tray-sealing machine (R145, Multivac, Germany). The oxygen transmission rates for top film and film for trays (Wipak multipet and Wipak Biaxer, Wipak, Nastola, Finland) were <1 cc/m²/24 h at 4°C and 50% relative humidity (for trays before forming). The modified atmosphere (MAP) contained 60% CO<sub>2</sub> and 40% N<sub>2</sub>. The packed chicken samples were stored in a chiller at 4°C for 19 d.

The pH of the leg meat was measured with a portable pH meter (Knick Portamess 911 Elektronische Messgeräte GmbH & Co. Berlin, Germany), by inserting an electrode in the center of the skinned and deboned thigh 24 h postmortem and the MAP stored samples on day 10 and 19.

### Color Measurements

Color of MAP packed samples was measured using CIELAB method, as lightness (L\*), redness (a\*), and yellowness (b\*) with Konica Minolta Spectrophotometer CM-700d (Konica Minolta Sensing Inc., Osaka, Japan) after 24 h, 10 and 19 d in MAP. The instrument measures wavelengths from 400 to 700 nm with 10 nm resolution. For the calculation of the three states of myoglobin (metmyoglobin, deoxymyoglobin, and oxymyoglobin), the method of Khatri et al. (2012) was used, as verified on a Minolta fiber instruments CM 700d (Slinde et al., 2019). The colorimeter was calibrated according to the manufacturer's instructions (Konica Minolta CM-700d Instruction Manual). Measurements were performed at 2 different locations on the surface of skinless thighs,

**Table 1.** Ingredient (%) and chemical composition (g/kg) of the experimental diets fed to chicken\*.

		Starter diet	ts (day 0-9)		Grower diets (day 10-30)				
Ingredients $\%$	$\mathrm{C}^1$	$\mathrm{CJ}10^2$	CJ20	CJ30	С	CJ10	CJ20	CJ30	
Wheat	52.8	53.6	54.5	55.3	62.5	63.1	63.7	64.3	
Oats	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	
C. jadinii <sup>a</sup>	0.0	4.9	9.8	14.7	0.0	4.2	8.3	12.5	
Soybean meal <sup>b</sup>	15.3	10.2	5.1	0.0	12.6	8.4	4.2	0.0	
Fish meal	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	
Rapeseed meal	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	
Potato protein concentrate <sup>c</sup>	5.0	5.0	5.0	5.0	3.0	3.0	3.0	3.0	
Maize gluten meal	5.0	5.0	5.0	5.0	2.0	2.0	2.0	2.0	
Soy oil	4.3	3.6	3.0	2.3	2.5	2.0	1.5	1.0	
Vitamin and trace-mineral mix <sup>d</sup>	0.64	0.63	0.63	0.63	0.62	0.63	0.63	0.63	
Limestone meal	0.95	0.95	0.92	0.91	0.8	0.79	0.78	0.77	
Monocalcium phosphate	0.67	0.78	0.88	0.99	0.46	0.55	0.63	0.72	
Sodium bicarbonate	0.41	0.33	0.24	0.16	0.4	0.31	0.23	0.14	
Sodium chloride	0.01	0.01	0.00	0.00	0.06	0.06	0.06	0.06	
L-Lysine	0.38	0.33	0.29	0.25	0.38	0.34	0.3	0.26	
L-Methionine	0.26	0.28	0.29	0.30	0.27	0.28	0.29	0.3	
L-Arginine	0.15	0.20	0.24	0.28	0.16	0.19	0.23	0.26	
L-Threonine	0.12	0.10	0.08	0.07	0.15	0.13	0.11	0.09	
L-Tryptophan	0.02	0.00	0.01	0.01	0.01	0.01	0.01	0.01	
Enzymes	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	
Titanium dioxide (TiO <sub>2</sub> )	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	
Calculated content									
Metabolizable energy <sup>e</sup>	2,892	2,892	2,892	2,892	2,820	2,820	2,820	2,820	
Crude protein	23.5	23.5	23.5	23.5	20.0	20.0	20.0	20.0	
Crude fat	59.4	57.4	55.5	53.5	42.5	39.2	35.8	32.5	
Crude fat: protein ratio	0.21	0.21	0.18	0.18	0.21	0.19	0.17	0.16	
Amino acids (%)									
Lysine	1.41	1.42	1.43	1.44	1.22	1.23	1.23	1.24	
Methionine	0.68	0.69	0.71	0.72	0.60	0.61	0.62	0.63	
Cysteine	0.37	0.36	0.34	0.32	0.37	0.32	0.30	0.29	
Threonine	0.95	0.95	0.95	0.96	0.83	0.83	0.83	0.83	
Tryptophan	0.27	0.27	0.27	0.27	0.24	0.24	0.24	0.24	
Arginine	1.45	1.46	1.46	1.47	1.27	1.27	1.27	1.27	

<sup>\*</sup>Experimental diet composition were obtained from Cruz et al. (2020).

and their average was determined. Each color value was analyzed by Spectra Magic Software (Minolta Inc., Japan).

#### **Texture Measurements**

Leg meat was kept in MAP and chilled at 4°C for 10 d until analysis. The meat was then vacuum packed, heated in a 80°C water bath to the end-point temperature of 74°C in the core of the sample. The internal temperatures of the samples were measured in dummy samples with a EBI-2T-313, 4 Channels log thermometer (Ing. Westad AS, Norway). Briefly after cooking, the samples were cooled down to room temperature and reweighed to determine cooking loss. Two strips  $(1 \times 1 \times 4 \text{ cm})$  were removed from the thigh and one from the drum  $(1 \times 1 \times 2.5 \text{ cm})$  by cutting parallel to the muscle fibers. Each strip was sheared perpendicularly to the fiber direction twice using shear cell HDP/BSK Warner Bratzler (load cell 25 kg, TA-HDi Texture Analyser, Stable Micro

Systems, Godalming, UK). The highest shear point was recorded and values are expressed in  $\rm N/cm^2$ .

## Fatty Acid Profile

The total fat content was extracted from 0.5 g of homogenized meat as described by Folch et al. (1957). For the extraction of fatty acids from meat, pure yeast flour, and the 5 starter and grower diets, the method described by O'Fallon et al. (2007) was used. The fatty acid methyl esters (FAME) analysis was carried out as reported by Inglingstad et al. (2017). Identification and quantification of FAME were based on external calibration with standard mixture ME100 (Larodan Fine Chemicals, Sweden).

#### Thiobarbituric Acid Reactive Substances

Development of lipid oxidation during storage was examined in MAP stored samples 24 h, 10, and 19 d. In

<sup>&</sup>lt;sup>1</sup>Control diets based on soybean meal, wheat, and oats (C) and formic acid (CA) had minimal differences in 0.6% formic acid addition to CA.

<sup>&</sup>lt;sup>2</sup>Diets with increasing levels of *C. jadinii* yeast replacing 10, 20, and 30% crude protein (CP) from SBM (CJ10, CJ20, and CJ30, respectively).

<sup>&</sup>lt;sup>a</sup>Dried inactivated C. jadinii (%): DM 97.0, CP 47.0, crude fat 1.6, ash 7.8, gross energy 4,756 kcal/kg; essential amino acid content in grams per 16g N: Arg 24.4, His 8.5, Ile 21.6, Leu 31.6, Lys 30.6, Met 5.2, Phe 18.4, Thr 25.6, Val 25.9, Trp 6.2.

<sup>&</sup>lt;sup>6</sup>Non-GMO soybean meal, Denofa AS, Norway: DM 877 g/kg, CP 457 g/kg, crude fat 22.8 g/kg, ash 57.3 g/kg, crude fiber 77 g/kg.

<sup>&</sup>lt;sup>c</sup>Cargil, Denmark.

 $<sup>^{</sup>m d}$ Vitamin-trace mineral premix, provided per 1 kg of diet: vitamin A 9600 IU; dl- $\alpha$ -tocopheryl acetate 100 mg; cholecalciferol 5000 IU; menadione 6 mg; thiamin 3.9 mg; riboflavin 7.4 mg; pantothenic acid 59 mg; niacin 20 mg; pyridoxine 12 mg; biotin 0.4 mg; cyanocobalamin 20  $\mu$ g; betaine 1.1 g; selenium 0.29 mg; Fe (FeSO<sub>4</sub>) 67 mg; Mn (MnO) 127 mg; Zn (ZnO) 60 mg; Cu(CuSO<sub>4</sub>) 11 mg; I (Ca [IO<sub>3</sub>]) 1.28 mg.

<sup>&</sup>lt;sup>e</sup>Apparent metabolizable energy, values in kilocalorie per kilogram, calculated based on Centraal Veevoederbureau (2005).

addition, lipid oxidation analysis was also performed on samples that were defrosted, preheated to 71°C, and stored at 4°C for 24 h to accelerate oxidation. Secondary lipid oxidation products were measured by the thiobarbituric acid reactive substance (TBARS) assay using the colorimetric method: previously homogenized meat was pulverized (IKA 11 basic Analytical mill, Germany) and 2 g mixed with 10 mL TBA stock solution (0.38 % TBA and 15% TCA in 0.25 N HCl). The tube was incubated for 10 min in a boiling water bath and thereafter cooled in water. The solution (1.5 mL) below the top fat layer was transferred to Eppendorf tubes and centrifuged (25 min at 21500  $\times$  g at 4°C). The absorbance of the solution was measured in duplicates at 532 nm (Synergy H4 Hybrid Microplate Reader BioTek, ThermoFisher, Göteborg, Sweden). TBARS value was calculated using the extinction coefficient of  $1.56 \times 10^5 / \mathrm{M}/$ cm and expressed as mg malondialdehyde (MDA)/kg of meat.

## Napping Sensory Test

The sensory analysis was performed as partial napping using 8 semitrained assessors. The focus was on odor, flavor, and texture. Two samples were replicated. Eight meat samples per dietary treatment were defrosted for 2 d at 2°C for sensory testing. Whole legs in vacuum bags were heated for 2 h at 70°C in an oven (Rational CombiMaster, mod CM 101) with steam (100% moisture). Fifteen min after cooking, the samples were sliced in  $3 \times 0.5$  cm pieces and served to the assessors. The serving temperature was  $30 \pm 5$ °C. The sensorv test was performed in a standardized sensory laboratory at Nortura (ISO 8589:2007). The recruited assessors (N = 8; 3 males and 5 females) were Nortura's employees, semitrained, but experienced in sensory evaluation (flavor and texture) of chicken meat using the partial napping method (Perrin et al., 2007). The assessors were asked to place the samples on an A3 paper according to perceived sensory difference, followed by an Ultra-Flash Profiling where the samples were described with sensory attributes (Perrin et al., 2008). X and Y coordinates were then recorded for each sample along with the frequencies of each attribute used to describe the samples.

# Thermal Desorption-Gas Chromatography-Mass Spectrometry Analysis of Volatile Compounds

Homogenized samples (meat, yeast, soybean meal) were weighed (2.5 g) in a glass vial (20 mL) with top cups and PTFE septa. Ten  $\mu$ L of a methanolic solution containing hexanoic acid ethyl ester at a concentration of 1.26 mg/g was used as an internal standard. HiSorb probes (Tenax TA/Carbograph) were conditioned on a TC-20 tube conditioner (Markes International Ltd., Liantrisant, UK) according to manufacturer instructions. HiSorb probe inserted in a headspace of the vial

was incubated at 35°C with agitation for 60 min using a HiSorb Agitator (Markes International Ltd., Liantrisant, UK). The HiSorb probe was removed from the vial, rinsed with MilliQ water, dried, placed in an empty tube, sealed with DiffLok caps, and analyzed within the day.

Under the same conditions, a mixture of pure compounds in Miglyol 812 (AXO INDUSTRY, Warve, Belgium) was processed as a control sample throughout the measurement period, at the beginning and end of sequences. The standard mix contained: butanal (99%), cis-2-penten-1-ol (95%), 2-undecanone (99%), dimethyl sulfone (98%), hexanal (98%), phenol (99.5%) (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany), acetic acid (100%, VWR, Fontenay-saus-Bois, France). Undecane (99%), decanoic acid ( $\geq$ 99.5%), 2-butoxy-ethanol ( $\geq$ 99.5%) (Sigma-Aldrich), 1,3-cyclopentanedione ( $\geq$ 97%, Fluka) and nonanal ( $\geq$ 99.5%, Sigma-Aldrich).

Volatile analyses were performed on an Automatic Thermal Desorption System AT100 (Markes, Llantrisant, UK) coupled with GC 6890N (Agilent Technologies, Wilmington, DE), DB-WAXetr fused silica capillary column (30 m  $\times$  0.25 mm i.d., 0.5  $\mu m$  film thickness; J&W Scientific; Agilent Technologies, Wilmington, DE) and ion source Agilent 5975. The method employed the desorption of volatiles at 280°C for 10 min with a column flow rate of 1.0 mL/min. The GC oven temperature was held at 35°C for 10 min, increased to 230°C at 10.3°C/min, and held for 10 min, Analysis lasted for 34 min and the recorded mass range was m/z 33 to 300. Semiquantification of all volatile compounds (VOC) was standardized to the calibration curve of chemicals in the standard mix.

## Extraction, Derivatization, and Gas Chromatography-Mass Spectrometry Analysis of Metabolites

Metabolite analysis was performed as previously described by Grabež et al. (2020). One g of homogenized meat (24 h postmortem) was mixed with a 5 mL solution of water: methanol: chloroform (1: 2.5: 1) and ribitol (66  $\mu g/mL$ ). The tube was heated in a sonication bath at 60°C for 60 min, centrifuged for 10 min at 3 000 rpm at 4°C, and 1 mL was transferred into an Eppendorf tube. The supernatant was dried in a SpeedVac (SPD11V-230, Thermo Scientific, Waltham, MA) overnight and stored at  $-80^{\circ}$ C until all samples were processed. The dried residues were dissolved in 80  $\mu$ L methoxyamine hydrochloride with pyridine (20 mg/mL) at 30°C for 60 min and sonicated at 30°C for 30 min. Finally, the samples were derivatized with 80 µL of N-methyl-N-(trimethylsilyl) trifluoroacetamide and incubated at 37°C for 30 min. An aliquot of 1  $\mu$ L was analyzed on a GC instrument from Thermo Fisher connected with a 1310-ISQ QD single quadrupole coupled with a capillary column (CP9012 VF-5ms 30m, ID 0.25 mm and 0.25  $\mu$ m film thickness with 5m EZ-Guard, Agilent). The GC temperature program was as following: 70°C for 5 min,

ramped at 5°C/min until 310°C. Analysis time was 60 min and the recorded mass range was m/z 50 to 700. The obtained MS files from the Chromeleon software v7.2 were translated to the netCDF format using Xcalibur v4.1.50. Furtherly, the GC/MS Agilent Translator created files to be used in MassHunter Qualitative Analysis vB.07.00 for processing. The NIST17 (National Institute of Standards and Technology/Gaithersburg, MD) and GOLM metabolome database (Max-Planck Institute for Molecular Plant Physiology, Golm, Germany) were used for metabolite identification. Compounds with  $\geq 70\%$  mass spectra match and presence in  $\geq 50\%$  of samples from the same dietary treatment were used for statistical analysis. Nine dilutions of the mixture were injected under the same conditions for the semi-quantification of identified metabolites. These compounds were: glycerol (85%) and glucose anhydrous (Merck, Darmstadt, Germany), succinic acid (99.5%), and methionine (98%) (Sigma Aldrich), myristic acid.

#### **Statistics**

ANOVA (GLM) was used to determine the effects diet on the production performance (SPSS statistics version 25, IBM Corp., Armonk, New York, 2017). Tukey's test (P < 0.05) was used for multiple comparisons between groups. The obtained data for carcass and meat quality characteristics, fatty acids, and metabolites were analyzed using one-way of variance (ANOVA) and Tukey's multiple comparison test (P < 0.05). ANOVA (GLM) was used to calculate the effects of storage time and diet on volatiles and TBARS. Fit regression model (Minitab, version 18 from Minitab Inc., State College, PA)  $y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \epsilon$  was applied to investigate the impact of the inclusion level of yeast  $(x_1,$  $x_2, x_3$  as predictor variables) on amino acids identified in chicken meat. Calculations of myoglobin states involved predictions from cross validated partial least square regression models, using scatter corrected data obtained on pure myoglobin states (Unscrambler X 10.5, Trondheim, Norway). Napping analyses was done using the multiple Procrustean factor analysis function of the SensoMineR package. The use of napping is easier than other sensory techniques to profile products although with impaired quantitation of differences (Lé et al., 2015).

#### RESULTS AND DISCUSSION

## **Dietary Composition**

The ingredient and chemical composition of experimental diets are shown in Tables 1 and 2, respectively. When replacing protein from SBM with yeast the soy oil in the diet was also reduced, due to the increased contribution of crude fat from yeast (Table 1). C. jadinii used in this trial had a crude fat content (16 g/kg DM) which was lower than the SMB (26 g/kg DM). Soy oil is rich in 18:2 (50-55%) according to the 2016, with 18:1 being the second most predominant fatty acid (21–25%), while

**Table 2.** Fatty acid profile (% by weight of total fatty acids) of yeast and grower diets.

		Controls		Yeast-containing diets				
Fatty acids	C. jadinii	С	CA	CJ10	CJ20	$\mathrm{CJ30}^1$		
14:0	0.10	0.32	0.37	0.28	0.38	0.35		
16:0	10.53	30.52	31.58	30.67	31.15	31.07		
18:0	3.85	5.21	5.17	6.17	4.95	4.83		
16:1n-7c	1.88	0.63	0.68	0.77	1.21	1.77***		
17:1n-7c	0.10	0.13	0.07	0.23	0.26	0.45**		
18:1n-9t	0.34	0.10	0.10	0.17	0.11	0.12*		
18:1n-9c	25.75	45.63	44.00	44.09	44.72	44.78**		
20:1n-9c	4.34	7.14	7.25	6.75	6.64	6.21**		
24:1n-9c	0.06	0.29	0.29	0.56	0.31	0.32		
18:2n-6c	41.45	0.09	0.09	0.11	0.13	0.18		
20:2n-6c	0.60	0.34	0.35	0.54	0.37	0.40		
20:3n-6c	0.26	0.05	0.06	0.13	0.06	0.05		
20:5n-3c	0.12	0.74	0.77	0.71	0.86	0.90		
22:6n-3c	0.04	1.08	1.17	1.17	1.20	1.39		

<sup>1</sup>Difference in fatty acids % between control and CJ30.

16:0 is present at 9% to 12% (Jokić et al., 2013). The yeast used in this study was also rich in 18:2 (41%), 18:1(26%), whereas 11% of 16:0 was present (Table 2). Most yeast strains are rich in monounsaturated fatty acids, such as 18:1n-9c (Alokla et al., 2014), while others are also rich in 18:2n-6. However, it is interesting to observe that yeast-based diets (starter and grower) had higher 18:2n-6 content (P < 0.01) than wheat-oat-SBM diets (not shown).

## Physicochemical Characteristics

The performance of broiler chicken was described by Cruz et al. (2020) regarding the inclusion levels of yeast to the diet (Table 3). The objective carcass and leg meat quality parameters of chicken fed the two wheat-oat-SBM-based diets (C and CA) and the three yeast-containing diets (CJ10, CJ20, and CJ30) are presented in Table 4. A decrease (P < 0.01) in cold carcass and total leg muscle weight was observed for the 30% yeast inclusion level, with an average reduction of 22.4% and 14.29\%, respectively, relative to birds fed the conventional control diet. These results coincide with Rodríguez et al. (2013) on the reduced carcass weight for the 30% yeast inclusion level. In a present study, an increased pellet hardness and temperature during feed manufacture was observed when the diets with a high level of yeast were produced (Cruz et al., 2020). In addition, the crude protein: crude fat ratio was confounded with yeast inclusion level (Table 1). Thus, the reduced performance of birds eating the diets with 20 and 30% of yeast inclusion (Table 3), could be related to increased pellet hardness and lower protein digestibility. Summarizing various studies, Vananuvat and Balloun (1977) reported poorer growth of broiler chicken when yeast replaced more than 15% to 20% of SBM or fish meal, suggesting that high levels of yeast (>15%) had an adverse effect on growth performance, but this needs further investigation.

<sup>(</sup>P < 0.001). \*\*(P < 0.01).

<sup>(</sup>P < 0.05).

**Table 3.** Overview of broiler chicken performance.\*

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	Controls		Y	east-containing diet	ts		
Item, $g/bird^1$	C	CA	CJ10	CJ20	CJ30	Pooled SEM	P-value
Start BW	42.1	42.2	41.6	41.8	42.2	0.15	
Final BW	$1987^{a}$	$2041^{a}$	$1935^{\mathrm{ab}}$	$1824^{bc}$	$1699^{c}$	35.77	< 0.001
BW gain							
Starter period	$246^{\mathrm{a}}$	$262^{\rm a}$	$239^{\mathrm{ab}}$	$226^{\mathrm{ab}}$	$188^{\rm b}$	13.38	0.010
Grower period	$1699^{a}$	$1737^{a}$	$1653^{\rm ab}$	$1555^{bc}$	$1469^{c}$	24.83	< 0.001
Overall	$1945^{a}$	$1999^{a}$	$1892^{ab}$	$1782^{bc}$	$1469^{c}$	35.75	< 0.001
Feed intake							
Starter period	275	283	288	296	254	11.43	
Grower period	2310 <sup>a</sup>	$2387^{a}$	$2257^{\mathrm{ab}}$	$2150^{bc}$	$2075^{c}$	31.15	< 0.001
Overall	$2540^{\rm ab}$	$2612^{a}$	$2500^{\mathrm{ab}}$	$2401^{bc}$	$2271^{c}$	37.81	< 0.001
$FCR^2$							
Starter period	$1.12^{\rm b}$	$1.08^{\rm b}$	$1.21^{\mathrm{ab}}$	$1.32^{a}$	$1.36^{a}$	0.04	< 0.001
Grower period	1.36	1.38	1.37	1.38	1.41	0.02	
Overall	$1.31^{\rm b}$	$1.31^{\rm b}$	$1.32^{ab}$	$1.35^{ab}$	$1.37^{a}$	0.01	< 0.01

<sup>\*</sup>Broiler chicken performance data were obtained from Cruz et al. (2020).

The pH of the leg meat was not systematically affected by diet. The highest inclusion level of C. jadinii (CJ30) gave lower (P < 0.01) cooking loss compared to CJ10 and CJ20 and the control. However, the reduction of cooking loss in CJ30 meat was 16% while the carcass weight was 21% compared with CJ10, therefore, having no practical relevance. Meat pH did not seem to be a predictor of cooking loss. Shear force was not dependent on diet, however, mean values for yeast containing-diets were numerically higher than those fed the conventional wheat-oat-SBM-based diets. The shear force was not affected by pH or cooking loss. The formic acid group had a lower (P < 0.01) cooking loss than the control group, as previously reported by Sugiharto et al. (2019). Thus, reduced cooking loss in CA meat provides greater product yield.

The substitution of SBM with different dietary levels of C. jadinii did not affect total intramuscular fat content in chicken leg meat. However, the changes in fatty acid profile in leg meat (Table 5) reflected the dietary reduction in soy oil, since the fat content in yeast was low. Increased 14:0 was observed with reduced soy: yeast ratio in the diets, which was possibly related to the reduction in total dietary lipids. The 18:1n-9t was the only fatty acid that logically may originate from yeast. In the yeast groups, the reduction of PUFA levels is consistent with the reduction of 18:2n-6 level in leg meat, no such trend was observed for 20:4n-6. Total saturated fatty acid (SFA) and monounsaturated fatty acids (MUFA) content increased, while polyunsaturated fatty acids (PUFA) decreased with increasing level of yeast in the diet. No difference in fatty acids was observed when formic acid was added to the diet.

Color stability is most easily accessed through increased metmyoglobin ( $\mathbf{MMb}$ ) fraction or reduced deoxymyoglobin ( $\mathbf{DMb}$ ) fraction of vacuum-packed leg meat. Already after 24 h, the CJ30 group revealed reduced (P < 0.01) color stability (Table 6). Reduced color stability was also observed in leg meat from the CJ20 and CJ30 groups after 10 d, whereas numerically lowest DMb was observed in samples from CJ30 group

after 19 d chilled storage. This indicated that leg meat color, based on DMb, was more stable in chicken fed SBM diets compared to the yeast-based diets. Antemortem and early postmortem factors such as stress and temperature, respectively, and vitamin E levels in feed have been reported affect color to meat (Faustman et al., 1998; Phung et al., 2013). However, small differences in vitamin E content were reported between yeast (<0.1 mg/100 g dry matter) (Matvaretabellen, 2020) and soybean meals (0.3 mg/100 g) (Banaszkiewicz, 2011). Thus, color stability is partly related to the postmortem mitochondrial activity and maintenance of reducing equivalents such as postmortem NADH concentrations. The lower glutamic acid in leg meat from yeast-containing diets (Table 7) and succinic acid (below level of quantitation) could have explained the color changes. When glutamic acid dominates over succinate, myoglobin stability is sensitive to other components affecting the Krebs cycle and electron transport chain, that is, a reduction in the succinate: glutamate ratio in meat leads to an increase in meat browning pending the presence of lipid oxidation products such as hexanal (Yi et al., 2015; Bjelanovic et al., 2016). The L\* value of leg meat from chickens fed a higher amount of yeast indicates that meat became less dark during chill storage; CJ30 had a higher (P < 0.05)L\* value than CA after 19 d chilled storage.

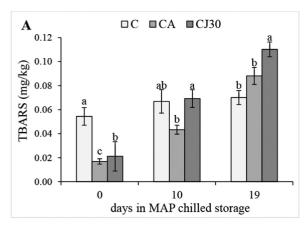
## Progression of Lipid Oxidation

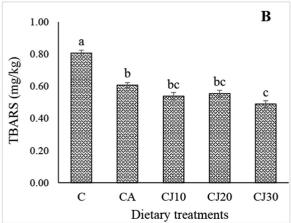
TBARS in fresh chill-stored leg meat were low, i.e. below 0.1 ppm (Figure 1A). One exception was the group CJ30 on day 19, that had a TBARS value of approximately 0.11 ppm. When the development in TBARS after chilled storage was compared between the control and CJ30 diets, the latter had a higher increase in TBARS indicating reduced oxidative stability. Zang et al. (2005a) reported reduced TBARS values in drumsticks after 10 d storage in birds fed Saccharomyces cerevisiae yeast-containing diet compared with those fed

<sup>&</sup>lt;sup>a-c</sup>Rows with different superscripts are statistically different (P < 0.05).

 $<sup>^{1}</sup>$ Values are presented as mean value of n=25 chicken per diet.

<sup>&</sup>lt;sup>2</sup>FCR, feed conversion ratio (g feed intake: g BW gain).





**Figure 1.** TBARS of chicken leg (A) following days of chilled storage under MAP and (B) after heating and 24 h chilling. Means with different superscripts (a, b, c) denote significant differences among dietary treatments (P < 0.05).

a corn-soybean meal diet. In this study, changes in TBARS after 19 d of chill-storage would not affect eating quality.

As pro- and antioxidants can be affected by heat treatments, oxidative stability was also compared when leg meat was heat treated and chilled (Figure 1B). A yeast-based diet reduced the level of oxidation caused by heat treatment, the CJ30 group showed lower TBARS compared with control diets (P < 0.05). The control with formic acid showed reduced TBARS in heat treated leg meat compared with control. However, oxidation (>0.5 mg/kg; Figure 1) that can be detected by a sensory panel (Gray and Pearson, 1987) was identified in all groups. Our results are in agreement with Xiong et al. (2020) that reported a 10-fold increase of TBARS in breast fillets heat treated at 70°C and stored for 2 d.

The most characteristic compounds of VOC profile of 24 h postmortem chicken leg meat were 16 highly branched alkanes (methylation  $\geq$  2). The number of identified branched alkanes declined to nine after 19 d of chilled storage (not shown). Few such compounds, such as 2,3-dimethyloctane were identified both at 24 h postmortem and on day 19. However, no relationship was found between the presence of 2,3-dimethyloctane and the dietary treatments on day 19. The number of branched alkanes in diets increased with the yeast level

(Table 8). Branched alkanes can be introduced in feed adding microbial ingredients such as yeast (Howard et al., 2013), but they are also present in plant ingredients (Kumari et al., 2014). C-methylation of branched alkanes to alkenes, lipids, and other molecules can be done by methyltransferases (Chen et al., 2020). The expression of these enzymes can be regulated through nutritional manipulation (Saande et al., 2019), which could support the hypothesis that yeast-containing feed will affect the expression of this enzyme in the broiler chicken. Higher level of the alkene 4,6-dimethylundecane was found in leg meat from chicken fed CJ20 and C30 compared to controls (P = 0.014). The fact that more branched alkanes were found in birds fed the CA diet may suggest a lack of degradation of alkanes to alkenes by the action of microbial enzymes, which has been idled by formic acid. Ayseli et al. (2014) measured volatiles in the raw chicken breast but did not report the presence of branched alkanes. Increased content of several linear alkanes such as hexane (not shown), octane, decane (not shown), and undecane (C6, C8, C10, and C11) was found after 19 d of chill storage. Undecane is a naturally occurring plant compound (Hunziker et al., 2015; Choi et al., 2020) and is also present in green algae (Sun et al., 2012). Decane and relatively high amounts of octane have recently been reported as yeast volatiles (Gonzalez et al., 2019). Octane and decane levels were higher in pure yeast meal than in soybean meal (P = 0.07; P < 0.001, respectively), whereas undecanewas not identified (not shown). Many alkanes have a faint petroleum odor regarded as unpleasant. However, their content was too low to have an impact on the leg meat flavor.

After 19 d of chilled storage, the aliphatic pentanoic, hexanoic, octanoic, nonanoic, and decanoic acids were identified in meat as free fatty acids, but only octanoic and decanoic acid contents were affected by diet (Table 8). Hexanoic and decanoic acids were also identified in pure soybean and yeast meal; both hexanoic acid (P = 0.033) and decanoic acid (P = 0.72) were numerically higher in the soybean meal. Ayseli et al. (2014) reported these acids as abundant volatiles in chicken breasts with decanoic acid as the most prevalent among the saturated fattv acids. In addition. Alasnier et al. (2000) found that with chilled storage of chicken thigh, the content of free fatty acid increased, while no further degradation was observed for aliphatic fatty acids. It is presumed that some aliphatic fatty acids originate from oxidative lipid degradation of longer unsaturated fatty acids. The present study showed higher content of aliphatic acids (i.e., octanoic, decanoic) in leg meat from controls compared with the yeast-containing groups.

Aldehydes and ketones are the most typical markers of oxidative degradation of polyunsaturated fatty acids. Six aldehydes (not shown) were detected in leg meat at day 19, but only octanal, pentadecanal, and benzaldehyde were affected by diet (Table 8). Thus, the level of aldehydes increased with chilled storage. Octanal and nonanal are degradation products of 18:1 and 18:2 fatty

acids, respectively (Morales et al., 1997; Belitz et al., 2004). At storage day 19, octanal, pentadecanal, and benzaldehyde contents were higher in the CA group compared with the C group (P < 0.05). This agrees with lipid oxidation measurements on day 19 that showed a numerically higher TBARS with formic acid addition to the feed. Octanal revealed small numerical differences between chicken fed the yeast-containing diets. On the other hand, the decrease of benzaldehyde and pentadecanal level in chicken fed the yeast-containing diets indicated possible improvement of oxidative stability in leg meat, although this is not supported by the nonanal contents. The analysis of pure yeast also showed lower (P <0.001) hexanal levels than the soybean meal. However, as soybean meal had higher lipid content, this may be the reason for its higher hexanal content. Benzaldehyde is another lipid oxidation-related compound generally present at too low levels to have an impact on flavor. The prevalence of aldehyde in leg meat produced by the CA diet indicated increased lipid oxidation. The level of two ketones (cyclopentane-1,2-dione and -hydroxybutan-2-one) increased (P < 0.05) when formic acid was added to the control but decreased with higher levels of yeast in the diets. Alghamdi et al. (2018) listed cyclopentane-1,2-dione as an antioxidative compound, although this is not clear from the results of the present experiment. The microbial metabolite 3-hydroxybutan-2-one had higher content than the CA-group (on day 19), indicating possible differences in terms of microorganisms on the meat surface. Another possible microbial compound such as dimethyl disulphide (not shown) had numerically higher content in the CA-group (P = 0.092). Other compounds appeared to be dependent on diet but did not seem directly related to any of the feed ingredients. Based on thresholds, the VOC results did not show a clear effect of diet on leg meat odor after 19 d storage.

# Changes in Meat Metabolites 24 h Postmortem as Affected by Chicken Diet

The untargeted metabolite analysis revealed 31 metabolites in chicken leg meat 24 h postmortem that were significantly (P < 0.05) affected by dietary treatment (Table 7). Metabolic changes in chicken leg meat caused by dietary changes, i.e. energy and nutrient

sources, showed differences not just between control with formic acid added and yeast-containing diets but also between the two control diets.

Formic acid added to the control diet showed a positive effect on the level of free amino acids in leg meat; higher glycine (P=0.005), serine (P=0.011), and total free amino acid (P=0.036) levels were found in the meat from CA. Formic acid in chicken feed showed a positive effect on feed utilization and protein digestibility by increasing the level of available energy and amino acids to the bird (Khodambashi Emami et al., 2013; Ndelekwute et al., 2015). The high growth rate of modern broiler chicken requires a high concentration of digestible proteins as well as essential amino acids (Qaisrani et al., 2020). Therefore, an increase of free amino acids in leg meat might indicate increased protein metabolism of the CA chicken muscle due to an increased rate of protein anabolism.

On the other hand, the substitution of SBM with an increasing level of yeast impaired chicken performance (Table 3) and affected free amino acid level in leg meat (Table 7). A linear increase of the essential amino acids was observed in leg meat with increased dietary levels of (P < 0.05). As previously reported by Cruz et al. (2020), reduced pellet quality with higher inclusion of yeast suppressed chicken feed intake (Table 3) and had a negative impact on general nutrient availability. An increase of free amino acids in leg meat of chicken fed with yeast-containing diets may indicate protein degradation in the muscle, as an adaptive response to reduced nutrient uptake or availability (Felig et al., 1969). Myofibrillar protein degradation in the chicken muscle is rather plausible keeping in mind a trend in loss of appetite and a small numerical increase in the mortality rate for chicken fed yeast-containing diets (Cruz et al., 2020), leading to reduced cold carcass weight for CJ30 compared with the controls (Table 4). In addition, the content of protein and amino acids in the chicken diet dictates free amino acid content in skeletal muscles (Watanabe et al., 2020). Therefore, even at the lowest yeast inclusion level (CJ10) protein metabolism in the chicken muscle may be affected. The content of all 11 free amino acids in leg meat was numerically lowest for the birds fed the CJ10 diet with the reduction of lysine considered as critical. Moreover, reduced taurine and elevated 2-hydroxybutyric acid content in chicken meat suggested higher oxidative stress and

 ${\bf Table\ 4.}\ {\bf Chicken\ carcass\ and\ leg\ meat\ quality\ characteristics.}$ 

	Controls		Ye	ast-containing di			
Item	$^{\mathrm{C}}$	CA	CJ10	CJ20	CJ30	Pooled SEM	P-value
Cold carcass weight (kg)	1.34 <sup>a,1</sup>	1.35 <sup>a</sup>	1.32 <sup>a</sup>	1.24 <sup>a</sup>	$1.04^{\rm b}$	0.01	< 0.01
Total leg muscle (kg)	$0.14^{a}$	$0.15^{a}$	$0.15^{a}$	$0.14^{a}$	$0.12^{\rm b}$	0.02	< 0.001
$IMF^2$ (%)	7.29	7.03	7.28	7.31	6.96	0.13	
pH 24 h	$6.45^{\mathrm{b}}$	$6.57^{ m ab}$	$6.50^{ m ab}$	$6.63^{a}$	$6.57^{\mathrm{ab}}$	0.02	< 0.01
$_{ m pH}$ 10 $\rm d_{MAP}$	6.15	6.15	6.12	6.19	6.14	0.01	
$_{ m pH}$ 19 ${ m d}_{ m MAP}$	$6.09^{\rm ab}$	$6.01^{\rm b}$	$6.06^{ m ab}$	$6.13^{a}$	$6.06^{\mathrm{ab}}$	0.01	< 0.01
Cooking loss (%)	$21.46^{a}$	$17.25^{bc}$	$19.45^{\rm ab}$	$19.76^{a}$	$16.25^{\circ}$	0.26	< 0.01
Max shear force (N/cm)	14.51	14.74	14.77	15.22	15.07	0.25	

 $<sup>^{\</sup>text{a-c}}\textsc{Rows}$  with different superscripts are statistically different (P < 0.01).

<sup>&</sup>lt;sup>1</sup>Cold carcass weight for C diet presents mean value of n = 17 chicken, and n = 20 for other diets.

<sup>&</sup>lt;sup>2</sup>Abbreviation: IMF = intramuscular fat content.

proteolysis with increased yeast in the diet. Metzler-Zebeli et al. (2019) reported a reduction of taurine, carnosine, and uric acid in the plasma due to enhanced proteolysis and the use of amino acids for gluconeogenesis as a response to restrictive feeding, which can also be consistent with loss of appetite.

Long-term impaired nutrient uptake stimulates lipolysis where triacylglycerides break down and release fatty acids needed to fuel the muscle with energy (Yaffee et al., 1980; Finn and Dice, 2006). With higher yeast levels in yeast-based chicken diets, the content of free fatty acids in leg meat increased, thus, small lipidderived molecules (i.e., 3-hydroxybutyric acid) that present an active energy source for the muscle (Newman and Verdin, 2014), numerically increased. In addition, an increase of malic acid with the yeast level in the chicken diet implies changes in the Krebs cycle. Organic acids increased in CA chicken leg meat. In CA meat 3-hydroxybutyric acid was significantly higher (P < 0.05) compared with the control. Also, CA meat had higher levels of metabolites involved in purine metabolism (hypoxanthine, inosine, and uracil) compared with the control that potentially reflects elevated tRNA for protein synthesis in vivo or AMP/IMP degradation early postmortem. On the other hand, the increasing levels of purine-derived compounds in chicken leg meat cooccur with increasing levels of yeast in the diets (Table 7). Previously, at low concentrations, nucleotiderich yeast extracts were reported as beneficial for short periods when included in feed for piglets (Van Buren and Rudolph, 1977) supporting the development of the gut, in addition to having an antimicrobial effect, but this was not evident in the present study.

# Nutritional Value of Chicken Meat Affected by Diet

Targeted fatty acid analysis (Table 5) and suspected untargeted metabolite analysis (Table 7) provide insight into the modulated nutritional value of chicken leg meat through dietary treatment. With yeast inclusion, the content of soy oil was reduced in chicken diets leading to an increase in MUFA and a decrease in PUFA level in leg meat. In general, reduced PUFA is undesirable, but the reduction of n-6/n-3 PUFA is regarded as beneficial from the public health perspective. However, chicken meat is a lean raw material and contributes little to the overall ingested fat in the Norwegian diet. In chicken leg meat originating from CA and CJ30 feed, the higher level of available amino acids is considered as improved protein quality, despite the fact that the high inclusion of veast reduced chicken growth. A similar effect of diet was found for vitamin B. Production of vitamin B occurs partly in the gut and is influenced by microbiota (Yoshii et al., 2019). Vitamin B3 is synthesized from tryptophan by intestinal bacteria and vitamin B5 is synthe sized from 2-dihydropantoate and  $\beta$ -alanine via de novo synthesis pathways (Yoshii et al., 2019), whereas vitamin B8 is generally not affected by gut microorganisms (Okazaki et al., 2018). A higher level of vitamin B5 in chicken leg meat is beneficial due to the fact that these

**Table 5.** Fatty acid profile (% by weight of total fatty acids) of chicken leg meat.

	Controls		Y	east-containing diet	S		
Item	C	CA	CJ10	CJ20	CJ30	Pooled SEM	P-value
14:0	0.51 <sup>b</sup>	0.51 <sup>b</sup>	$0.52^{\rm b}$	$0.54^{\rm ab}$	$0.56^{\rm a}$	0.008	< 0.001
16:0	$22.26^{b}$	$22.56^{\mathrm{ab}}$	$22.89^{ab}$	$23.17^{a}$	$23.30^{a}$	0.192	0.001
18:0	6.52	6.63	6.54	6.46	6.65	0.104	
14:1n-5c	$0.14^{c}$	$0.14^{c}$	$0.16^{bc}$	$0.16^{\mathrm{ab}}$	$0.18^{a}$	0.005	< 0.001
16:1n-7c	$4.40^{c}$	$4.41^{c}$	$4.77^{bc}$	$5.14^{\mathrm{ab}}$	$5.53^{a}$	0.118	< 0.001
18:1n-9t	$0.20^{c}$	$0.21^{bc}$	$0.22^{bc}$	$0.23^{ab}$	$0.25^{a}$	0.005	< 0.001
18:1n-9c	$32.41^{\rm b}$	$32.38^{b}$	$33.63^{ab}$	$34.47^{a}$	$34.38^{a}$	0.347	< 0.001
20:1n-9c	1.62 <sup>a</sup>	1.60 <sup>a</sup>	$1.52^{\rm b}$	$1.45^{\rm b}$	$1.34^{c}$	0.016	< 0.001
22:1n-9c	0.06	0.06	0.06	0.06	0.06	0.002	
18:2n-6c	$26.41^{a}$	$26.03^{a}$	$24.47^{\rm b}$	$23.28^{bc}$	$22.24^{c}$	0.320	< 0.001
18:3n-6c	0.19	0.18	0.17	0.18	0.19	0.006	
18:3n-3c	$1.36^{a}$	$1.34^{a}$	$1.27^{\rm b}$	$1.22^{\mathrm{b}}$	$1.12^{c}$	0.014	< 0.001
20:2n-6c	0.37	0.39	0.35	0.34	0.35	0.014	
20:3n-3c	0.03	0.03	0.03	0.03	0.03	0.001	
20:3n-6c	$0.29^{\mathrm{ab}}$	$0.31^{ab}$	$0.28^{\rm b}$	$0.28^{\rm b}$	$0.34^{\rm a}$	0.014	0.026
20:3n-9c	$0.08^{\rm b}$	$0.08^{\rm b}$	$0.08^{\rm b}$	$0.09^{b}$	$0.12^{a}$	0.014	< 0.001
20:4n-6c	1.82	1.80	1.72	1.60	1.86	0.112	
20:5n-3c	$0.20^{\rm b}$	$0.20^{\rm b}$	$0.20^{\rm b}$	$0.20^{\rm b}$	$0.24^{\rm a}$	0.008	0.04
22:6n-3c	0.57	0.59	0.58	0.56	0.67	0.037	
$\sum SFA^1$	$29.76^{c}$	$30.18^{bc}$	$30.43^{ m abc}$	$30.65^{\rm ab}$	$31.02^{a}$	0.083	< 0.001
∑MUFA	$38.84^{bc}$	$38.80^{c}$	$40.34^{\rm ab}$	$41.51^{a}$	$41.74^{\rm a}$	0.174	< 0.001
∑PUFA	$31.37^{a}$	$30.99^{a}$	$29.20^{\rm b}$	$27.82^{bc}$	$27.22^{c}$	0.194	< 0.001
n-3	0.81	0.83	0.81	0.79	0.95	0.020	
n-6	$30.45^{\rm a}$	$30.05^{a}$	$28.27^{\rm b}$	$26.90^{bc}$	$26.10^{\circ}$	0.178	< 0.001

 $<sup>^{1}</sup>$ ∑SFA (saturated fatty acids): sum of 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, and 23:0; ∑MUFA (monounsaturated fatty acids): sum of 14:1n-5c, 16:1n-7c, 18:1n-9t, C18:1n-9c, C20:1n-9c; ∑PUFA (polyunsaturated fatty acids): sum of C18:2n-6c, C18:3n-6c, C18:3n-3c, C20:2n-6c, C20:3n-3c, C20:3n-6c, C20:3n-9c, C20:4n-6c, C20:5n-3c, and C22:6n-3c.

<sup>&</sup>lt;sup>a-c</sup>Rows with different superscripts are statistically different (P < 0.05).

Table 6. Color characteristics of chilled stored chicken leg meat in MAP.

		Con	trol	Y	east-containing die	ets		P-value
State Time (day)	Time (day)	С	CA	CJ10	CJ20	CJ30	Pooled SEM	1 varac
OMb	1	$0.05^{y}$	$0.07^{y}$	$0.06^{y}$	$0.08^{z}$	$0.08^{y}$	0.006	
	10	$0.03^{y}$	$0.02^{y}$	$0.02^{y}$	$0.02^{y}$	$0.03^{y}$		
	19	$0.14^{x}$	$0.18^{x}$	$0.14^{x}$	$0.16^{x}$	$0.20^{x}$		
DMb	1	$0.93^{a,x}$	$0.90^{\rm a,x}$	$0.92^{\mathrm{a,x}}$	$0.90^{a,x}$	$0.80^{\rm b,x}$	0.010	< 0.01
	10	$0.83^{ m ab,x}$	$0.86^{a,x}$	$0.83^{\rm ab, x}$	$0.80^{\rm b,x}$	$0.81^{\rm ab, x}$		< 0.01
	19	$0.58^{y}$	$0.54^{y}$	$0.56^{y}$	$0.51^{y}$	$0.51^{y}$		
MMb	1	$0.02^{\mathrm{a,y}}$	$0.02^{a,y}$	$0.03^{a,y}$	$0.02^{a,y}$	$0.11^{b,y}$	0.006	< 0.01
	10	$0.28^{x}$	$0.28^{x}$	$0.30^{x}$	$0.33^{x}$	$0.29^{x}$		
	19	$0.28^{x}$	$0.28^{x}$	$0.30^{x}$	$0.33^{x}$	$0.29^{x}$		
$L^*$	1	54.24	54.02	54.90	54.87	55.04	0.210	
	10	54.96	54.78	54.83	56.31	55.08		
	19	$55.54^{ m ab}$	$54.83^{\rm b}$	$54.90^{ab}$	$56.06^{\mathrm{ab}}$	$56.36^{a}$		< 0.05
$a^*$	1	-0.61	-0.64	-0.42	-0.35	-0.57	0.189	
	10	0.11	-0.33	0.02	-0.40	-0.33		
	19	0.43	0.05	-0.10	-0.02	0.05		
$b^*$	1	8.27	8.50	9.31	9.16	8.84		
	10	$8.46^{ m ab}$	$7.90^{\rm b}$	$8.96^{\mathrm{ab}}$	$9.41^{a}$	$8.72^{ab}$	0.331	< 0.05
	19	9.18	8.36	9.24	9.03	8.79		

Table 7. Dietary effect on metabolites (mg/kg) identified in chicken leg meat.

		Con	trol	Ye	ets		
RT (min)	Metabolites	C	CA	CJ10	CJ20	CJ30	Pooled SEM
Amino acids							
11.71	Alanine	$2254^{\mathrm{ab}}$	2759 <sup>a</sup>	$1803^{\rm b}$	$2128^{ab}$	$2629^{\mathrm{ab}}$	105
15.20	Valine	$485.10^{ab}$	618.10 <sup>a</sup>	$307.10^{\rm b}$	$475.40^{ab}$	$613.50^{a}$	29.3
17.47	Isoleucine	$212.30^{ab}$	273.70 <sup>a</sup>	$130.50^{\rm b}$	$217.0^{ab}$	$269.20^{\rm a}$	13.4
17.82	Glycine	$2878^{\rm b}$	4017 <sup>ab</sup>	$2918^{\rm b}$	$3994^{ab}$	4356 <sup>a</sup>	14.9
19.33	Serine	1861 <sup>b</sup>	$2945^{\mathrm{ab}}$	$1908^{\rm b}$	$2282^{\mathrm{ab}}$	$3092^{a}$	1278
20.00	Threonine	$2080^{\mathrm{ab}}$	2815 <sup>a</sup>	$1506^{\rm b}$	$2070^{ab}$	2548 <sup>a</sup>	121
23.49	aspartic acid	$1437^{ab}$	$1652^{ab}$	1177 <sup>b</sup>	$1412^{\mathrm{ab}}$	1842 <sup>a</sup>	76.4
25.89	Glutamic acid	2433 <sup>ab</sup>	3052 <sup>a</sup>	1816 <sup>b</sup>	$2052^{\rm b}$	$2663^{ab}$	201
26.03	Phenylalanine	315.9 <sup>ab</sup>	425.8 <sup>a</sup>	$204.3^{\rm b}$	294.6 <sup>ab</sup>	$367.5^{ab}$	19.5
32.33	Lysine	$1628^{ab}$	2152 <sup>ab</sup>	1313 <sup>b</sup>	1913 <sup>ab</sup>	2370 <sup>a</sup>	89.6
32.70	Tyrosine	$520^{\mathrm{ab}}$	721.9 <sup>a</sup>	$373.4^{\rm b}$	515.1 <sup>ab</sup>	710.7 <sup>a</sup>	37.0
Total	1,1001110	14477.9 <sup>ab</sup>	19180.4 <sup>a</sup>	12143.6 <sup>b</sup>	15439.7 <sup>ab</sup>	19091.6 <sup>a</sup>	3108.6
13.89	2-aminobutanoic acid	11.2 <sup>ab</sup>	11.2 <sup>ab</sup>	9.10 <sup>a</sup>	10.8 <sup>ab</sup>	15.1 <sup>a</sup>	0.55
21.14	ß-alanine	$2282^{bc}$	3634 <sup>a</sup>	1483 <sup>c</sup>	$2217^{bc}$	3043 <sup>ab</sup>	105
22.20	2-aminomalonic acid	$47.9^{\rm b}$	218.3 <sup>a</sup>	49.4 <sup>b</sup>	$129.7^{ab}$	147.2 <sup>a</sup>	11.3
22.70	Malic acid	268.2 <sup>b</sup>	469.3 <sup>a</sup>	177.2 <sup>b</sup>	236.8 <sup>b</sup>	$346.1^{ab}$	19.9
23.56	Pyroglutamic acid	$2037^{ab}$	2937 <sup>a</sup>	1719 <sup>b</sup>	$2024^{\mathrm{ab}}$	$2538^{ab}$	133
26.93	Taurine	$329.5^{ab}$	488 <sup>a</sup>	$322^{ab}$	178.8 <sup>ab</sup>	100 <sup>b</sup>	40.6
Small organic		020.0	100	022	110.0	100	10.0
12.46	2-hydroxybutyric acid	$9.11^{ab}$	$12.41^{ab}$	$7.97^{\rm b}$	$10.42^{ab}$	$12.56^{\rm a}$	0.53
13.55	3-hydroxybutyric acid	$67.10^{b}$	153.50 <sup>a</sup>	45.60 <sup>b</sup>	84.10 <sup>b</sup>	93.60 <sup>b</sup>	6.11
15.87	4-hydroxybutanoic acid	10.80 <sup>b</sup>	17.60 <sup>a</sup>	$12.30^{ab}$	13.10 <sup>ab</sup>	$13.40^{\rm ab}$	0.62
Nucleotide rel		10.00	11.00	12.00	10110	10.10	0.02
18.74	Uracil	$66.3^{\mathrm{ab}}$	$98.2^{a}$	$52.2^{\mathrm{b}}$	$69.6^{\mathrm{ab}}$	$93.5^{a}$	4.0
30.09	Hypoxanthine	196.2 <sup>ab</sup>	294.4 <sup>a</sup>	154.6 <sup>b</sup>	$219.9^{ab}$	294.5 <sup>a</sup>	14.1
43.69	Inosine	822.6 <sup>b</sup>	1270 <sup>a</sup>	$734.9^{b}$	$995^{\mathrm{ab}}$	1418 <sup>a</sup>	50.9
Short sugar	111001110	022.0	12.0	101.0	000	1110	00.0
18.51	Glyceric acid	$17.1^{ab}$	$22.5^{\mathrm{a}}$	$13.5^{\rm b}$	$12.8^{\rm b}$	$18.3^{ab}$	0.9
Fatty acids		-,					0.0
34.87	Palmitic acid	$827^{\mathrm{bc}}$	$1369^{a}$	$625^{c}$	$937^{ m abc}$	$1313^{ab}$	63.1
38.43	Stearic acid	582.8 <sup>ab</sup>	795 <sup>a</sup>	$428.4^{\rm b}$	$578.4^{ab}$	854.4 <sup>a</sup>	39.6
40.53	Arachidonic acid	$72.4^{ab}$	$58.8^{\mathrm{ab}}$	$42.7^{\rm b}$	$48.6^{\mathrm{ab}}$	98ª	6.0
Phospho-lipid		· <del>-</del> · -					
29.44	Phosphoryletanolamine	$144.1^{bc}$	239.1 <sup>a</sup>	$96.7^{c}$	$142.4^{bc}$	$196.8^{ab}$	11.1
Vitamins B			200.1			100.0	
22.64	Niacinamide, B3	61.1 <sup>b</sup>	94.1 <sup>a</sup>	$52^{\rm c}$	$68^{ m abc}$	$85.8^{\mathrm{ab}}$	3.2
33.71	Pantothenic acid, B5	$22.8^{\rm b}$	50.0 <sup>a</sup>	24.3 <sup>a</sup>	27.1 <sup>a</sup>	$32.7^{ab}$	2.2
35.46	Myo-inositol, B8	1089.3 <sup>ab</sup>	1404 <sup>a</sup>	854.5 <sup>b</sup>	$1026.2^{\rm b}$	1155.4 <sup>ab</sup>	42.0

 $<sup>^{\</sup>text{a-c}}\textsc{Rows}$  with different superscripts are statistically different (P  $\leq 0.05).$ 

 $<sup>^{\</sup>rm a-c}$ Rows with different superscripts are statistically different. Abbreviations: DMb, deoxymyoglobin; L\*, lightness; MMb, metmyoglobin; OMb, oxymyoglobin.

Table 8. TD-GC/MS identified volatile compounds (mg/kg) in chicken leg meat chill stored in MAP.

		Controls Yeast-containing diets		diets		Odor threshold			
RT (min)	Volatile compound	$^{\mathrm{C}}$	CA	CJ10	CJ20	CJ30	Pooled SEM	range in mixtures $(mg/kg)^1$	Odor/taste description
Effect of die	et on fresh meat (day 0)								
9.56	4,6-dimethylundecane	$0.001^{\rm b}$	$0.043^{a}$	$0.001^{\rm b}$	$0.072^{a}$	$0.073^{a}$	0.010	n.a.	n.a.
10.41	Undecane	$0.007^{bc}$	$0.026^{\rm ab}$	$0.001^{c}$	$0.041^{a}$	$0.045^{a}$	0.006	5.8	Alkane (1)
11.47	2,3-dimethyloctane	$0.001^{\rm b}$	$0.019^{a}$	$0.002^{\rm b}$	$0.029^{\rm a}$	$0.033^{a}$	0.004	n.a.	n.a.
7.68	2-ethylhexyl hexyl sulfite	$0.008^{bc}$	0.016 <sup>ab</sup>	$0.002^{c}$	0.018 <sup>ab</sup>	0.025 <sup>ab</sup>	0.002	n.a.	n.a
8.87	$\delta$ -3-carene	< 0.001 <sup>b</sup>	$0.007^{\mathrm{b}}$	< 0.001 <sup>b</sup>	$0.004^{\rm ab}$	$0.008^{\rm a}$	0.003	0.77	Bell pepper, lemon, pungent, resin, rubber (2)
20.76	2-butoxyethanol	$0.264^{\rm b}$	$0.299^{\rm ab}$	$0.712^{a}$	$0.382^{ab}$	$0.223^{\rm b}$	0.106	n.a.	Sweet, ether-like, ran- cid, pleasant odor (3)
Effect of die	et on chilled stored meat in	(							
3.42	Octane	0.007 <sup>ab</sup>	0.013 <sup>b</sup>	0.005 <sup>b</sup>	0.007 <sup>b</sup>	0.031 <sup>a</sup>	0.005	0.94	Alkane, fat, oil, sweet (4)
10.40	Undecane	$0.316^{ab}$	$0.424^{a}$	$0.356^{\rm b}$	$0.325^{\rm b}$	$0.292^{\rm b}$	0.022	5.8	Alkane (5)
14.22	3-hydroxybutan-2-one	$0.016^{\rm b}$	0.050 <sup>a</sup>	0.025 <sup>ab</sup>	$0.010^{\rm b}$	$0.005^{b}$	0.010	6-10 (6)	Butter, cream, green, pepper, rancid, sweat (7)
23.58	Octanoic acid	$5.49^{ab}$	9.15 <sup>a</sup>	$5.86^{\mathrm{ab}}$	$3.90^{\rm b}$	$3.61^{\rm b}$	0.991	10-15	Acid, cheese, fat, rancid, sweat (7)
25.72	Decanoic acid	$22.40^{\rm b}$	$84.6^{\rm \ a}$	$33.9^{\mathrm{ab}}$	$21.5^{\ b}$	17.3 <sup>b</sup>	7.200	3-15	Dust, fat, grass, rancid, sweat (7)
Storage (da	y 0 and 19 in MAP) and d	iet effect <sup>2</sup>							(,)
14.101	Octanal	n.d	n.d	n.d	n.d	n.d		0.2 - 0.6	Citrus, fat, green, nut,
		$0.980^{a}$	$1.130^{a}$	$1.050^{\rm a}$	$1.060^{a}$	$1.120^{\rm a}$	0.099		pungent $(7)$
15.671	Nonanal	$0.059^{a,x}$ $0.825^{b,y}$	$0.075^{a,x}$ $1.060^{b,y}$	$0.148^{a,x}$ $0.751^{b,y}$	$0.079^{a,x}$ $0.601^{y}$	$0.084^{a,x}$ $0.863^{b,y}$	0.325	0.01 - 5	Fat, green, paint, citrus, pungent citrus (7)
17.757	Benzaldehyde	$0.017^{a}$	$0.019^{a}$	$0.019^{a}$	$0.017^{a}$	$0.018^{a}$		0.2 - 5	Bitter almond, burnt
	v	$0.058^{\rm b}$	$0.341^{c}$	$0.069^{\rm b}$	$0.061^{\rm b}$	$0.037^{\rm b}$	0.015		sugar, cherry, malt (7)
24.258	Pentadecanal	$^{ m n.d}_{0.045^{ m b}}$	n.d 0.141 <sup>a</sup>	$^{ m n.d}_{ m 0.080^a}$	$^{ m n.d}_{0.037^{ m b}}$	$^{ m n.d}_{ m 0.029^{b}}$	n.d 0.012	n.a.	Fresh, waxy (8)

<sup>&</sup>lt;sup>a-c</sup>Rows with different superscripts are statistically different ( $P \le 0.05$ ).

nutrients are marginal in the diet of young Norwegian women (Egelandsdal et al., 2020) who also prefer this type of meat.

## Sensory Characteristics

Using napping test to evaluate sensory properties of chicken leg meat, the acquired descriptors were fresh and mild for odor, and for taste fresh, acid, egg/sulfur, umami, and metallic. Although the napping procedure did not provide clear differences in sensory profiles between diet groups (not shown), meat originating from chicken fed yeast-containing diets was more frequently described as metallic. Metallic flavor has been related to secondary lipid oxidation compounds (Jayasena et al., 2013). In addition, the bitter flavor of heat-treated meat was perceived by the panelists, with an apparent link to the use of yeast in the chicken diet. Hypoxanthine is a potential marker of bitterness that results from inosine degradation. This reduces the umami flavor which is a desirable characteristic of meat. However, the taste threshold of hypoxanthine is matrix dependent and it appears that the level in the CA diet would be at the limit of detection (Toldrá, 2008).

#### CONCLUSION

The addition of *C. jadinii* yeast (4.9–14.7%) in broiler-chicken diets reduced carcass weight and n-6 fatty acids compared with a soybean-based diet added 0.6% formic acid. Additionally, the warmed-over flavor was reduced for the chicken fed diets where 30% of the protein in the soybean-based diet was replaced with yeast. Lipid derived volatiles supported an increase of oxidative stability in chill-stored leg meat with higher yeast levels in chicken diets. The addition of yeast in the chicken diet affected metabolites, as indicators of nutrient uptake, in leg meat. A soybean-based diet added 0.6% formic acid reduced cooking loss and provided a better nutritional value of chicken leg meat, that is, vitamin B3 and B5.

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<sup>&</sup>lt;sup>x.y</sup>Columns with different superscripts are statistically different  $(P \le 0.05)$ .

<sup>&</sup>lt;sup>1</sup>If no reference in the cells, the relevant reference is presented under the odour description:(1) Champagne and Nawar (1969), (2) Tamura et al. (1996), (3) NIH (2020a), (4) García-González et al. (2008), (5) NIH (2020b), (6) Rothe and Thomas (1963), (7) VCF (2020), (8) TGSC-Information-System.

<sup>&</sup>lt;sup>2</sup>GLM used; n.a., not available; n.d., not detected.

compounds. The work was funded by Foods of Norway, Centre for Research-based Innovation (Research Council of Norway; grant 237841/030) project.

#### **DISCLOSURES**

All authors declare that they have no conflict of interest.

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