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Cyberlindnera jadinii yeast as a protein source for growing pigs: Effects on protein and energy metabolism

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ABSTRACT

Inactivated *Cyberlindnera jadinii* yeast (previously classified as *Candida utilis*) produced from local lignocellulosic biomass-based sugars is an alternative protein source in diets for young pigs. The objective of this study was to evaluate the effects of diets containing *C. jadinii* on the nitrogen and energy metabolism and apparent total tract digestibility (ATTD) of major nutrients and energy in young pigs. Twenty-four intact boars with a mean initial body weight of 16.7 ± 4.5 kg were assigned to four diets: a conventional control diet for young pigs with soybean meal, fish meal, rapeseed meal and potato protein concentrate as major protein sources or one of three experimental diets containing 10, 20, and 40%% of crude protein (CP) from *C. jadinii*. The pigs were equally distributed to the dietary treatments according to initial body weight and litter, comprising a total of six replicates per diet. The experiment was carried out during three periods of 14 days. Each period comprised an energy and nitrogen balance experiment of four consecutive days including a 22 h respiration experiment by means of indirect calorimetry. Adding *C. jadinii* to diets did not affect the ATTD of nutrients and energy in the diets. The energy and nitrogen metabolism was not affected by partially replacing the main protein sources with *C. jadinii*. Collectively, the results indicate that CP from *C. jadinii* can replace up to 40% of dietary CP from conventional protein sources while maintaining the efficiency of nitrogen and energy metabolism in young pigs.

1. Introduction

Protein production in Europe is currently insufficient to satisfy the demands of the livestock sector (de Visser et al., 2014; Roman et al., 2016). In addition, the prices of locally produced protein-rich ingredients offer little competition to those available through imports, leading to a heavy reliance on imported protein sources, such as soybean meal (SBM) (Tallentire et al., 2018; van Krimpen et al., 2013). Identifying alternative ingredients is, therefore, crucial to maintaining sustainability in agriculture. The use of new technology allows the production of yeast from local non-food biomass that does not compete directly with human food, offering potential to improve self-sufficiency and sustainability (Schader et al., 2015; Øverland and Skrede, 2017). *Cyberlindnera jadinii* (previously classified as *Candida utilis*) grown on sugars from lignocellulosic biomass, has high crude protein (CP) with a favorable amino acid composition, resembling high-quality protein sources such as SBM. Yeasts are additionally rich in proteins, mannans, β-glucans (Kogan and Kocher, 2007; Kollár et al., 1997) and nucleotides (Bacha et al., 2013; Halász and Lásztity, 1991), which can improve growth rate and gut function in young pigs (Cruz et al., 2019; Mateo et al., 2004; Mateo and Stein, 2004; Shurson, 2018). Yeast is commonly used as a feed additive (< 1% of the formulation), but less information is available on yeast as a potential protein source in pig diets. In comparison, fish meal (FM) is a high-quality protein source commonly used in Europe, however, the availability of this resource is limited (Hardy, 1996; Tallentire et al., 2018). Furthermore, rapeseed meal (RSM) is partially available in northern Europe but has a lower nutritional value in terms of CP and essential amino acids (Van Zanten et al., 2015) and a higher content of fiber and glucosinolates compared with SBM (Mejicanos et al., 2016; Pérez de Nanclares et al., 2017). Field beans and peas may also be used in conventional diets but they have lower CP (22 to 38%) compared to conventional protein sources (Griffiths and Lawes, 1978) and are associated with antinutritional factors (Moseley and Griffiths, 1979).

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Currently, limited information exists about the utilization of energy and nitrogen (N) of pigs fed diets with moderate inclusion levels of *C. jadinii, i.e.* 10 to 40% of the dietary CP. Energy balance measurements are valuable to evaluate for newly introduced feed ingredients (Noblet, 2007) and N balance measurements provide valuable information about the utilization of dietary protein for retention in body tissues. We wanted to evaluate if protein and energy from *C. jadinii* could be equally utilized by pigs as those provided by conventional high-quality protein sources. Therefore, we hypothesized that *C. jadinii* is a viable alternative protein source that can replace up to 40% of the CP from conventional protein sources in diets for young pigs while N and energy utilization, growth and digestive performance are maintained. The objectives of this study were, thus, to evaluate the effects of diets containing up to 40% of CP from *C. jadinii,* which replaced the main protein sources in pig diets by measuring 1) the N and energy metabolism and 2) the apparent total tract digestibility (ATTD) of major nutrients and energy among pigs.

2. Materials and methods

All animals were handled and cared for in accordance with the Animal Welfare Act of 28 December 2009, and the research protocol complied with the guidelines of The Animal Experiments Inspectorate, Ministry of Environment and Food, Copenhagen, Denmark, regarding animal experimentation and care (license no. 201515-0201-00,685).

2.1. Animals and facilities

Twenty-four crossbred-intact boars (Danish Landrace × Yorkshire) were obtained from a commercial herd and housed at the experimental facilities at Rørrendegård experimental farm, Taastrup, Denmark. The experiment was carried out with four experimental diets during three consecutive periods, with two pigs per diet per period and a total of six replicates per diet. Each animal was fed and weighed individually and thus each pig constituted an experimental unit. Each set of piglets per period comprised two sets of four litter-mates. Each litter-mate was allotted to one of the four dietary treatments. The piglets were equally distributed to the dietary treatments according to initial body weight (BW). The three periods were divided into a seven-day period of adaptation in stables (where the animals adapted to the experimental facilities and diets) followed by a three-day adaptation period in metabolism cages, which preceded the balance and respiration experiments. Each balance period lasted for four days and it comprised an energy and N balance experiment of four consecutive days including a 22-h respiration experiment by means of indirect calorimetry. Each two pigs entered the two respiration chambers on Mondays at 1100 h and exited on the next day at 0900 h. The next two pigs entered the chambers on Tuesdays at 0900 h, and so on. Thus, in each of the three balance periods, eight pigs were individually submitted to respiration measurements from Monday to Friday. The BW was measured individually at four time-points: at the start of the adaptation period (day 1), at the end of the adaptation period (day 7), at the start of the balance period (day 11) and at the end of the balance period (day 15). Mean BW at the beginning of the adaptation period was (mean ± standard deviation) 16.69 ± 4.45 kg and the pigs were on average 54 ± 4.3 days old. Straw bedding was provided to ensure the comfort of the animals during the adaptation period in pens, while during the balance period, rubber mats were placed inside the metabolism cages. Environment enriching toys were available for each pig throughout the experiment. Fecal score was registered daily and for each pen during the adaptation period and for each pig after the pigs were transferred to the metabolism cages, on a scale from 1 to 4, according to consistency and shape (1 = dry and hard; 2 = normal "sausage-shaped"; 3 = loose stools, pasty consistency with some loss of normal shape; 4 = watery diarrhea with complete loss of normal shape) to monitor the occurrence of diarrhea and loose stools (fecal score ≥ 3) (Pedersen and Toft, 2011). Room temperatures (°C) in the adaptation stables and the metabolism room were 17.6 ± 0.97 and 20.7 ± 1.42, respectively.

2.2. Diets and feeding

Cyberlindnera jadinii (LYCC 7549; Lallemand Yeast Culture Collection) was obtained from Lallemand Inc, Salutaguse, Estonia. Sugars used in the growth media for *C. jadinii* were obtained from lignocellulosic biomass of Norway spruce trees (*Picea abies*) by using the BALI (Borregaard Advanced Lignin) process at Borregaard AS, Sarpsborg Norway (Patent "Lignocellulosic biomass conversion by sulfite pretreatment"; EP2376642B1 EP Grant) as described by Øverland and Skrede (2017) and Sharma et al. (2018). *C jadinii* was inactivated and drum-dried prior to being included in the diets.

The dietary treatments consisted of one control diet and three diets containing 3.6%, 7.3% and 14.6% of *C. jadinii*, which partially replaced conventional protein sources: fishmeal, rapeseed meal, soybean meal, and potato protein concentrate. Crude protein (CP) in the diets was replaced at the rates of 0, 10, 20 and 40% respectively, based on the standardized ileal digestibility (SID) values of CP and of essential amino acids (AA) for pigs in the conventional feedstuffs. The SID of CP and AA for *C. jadinii* was estimated based on the SID of CP for brewer's yeast (*Saccharomyces cerevisiae*) in pigs (Centraal Veevoederbureau, 2005). All diets were thus formulated to be isonitrogenous and isoenergetic and to meet or exceed pig nutritional requirements for energy, amino acids, and all other nutrients. The diets (Table 1) were formulated by Felleskjøpet Fôrutvikling, A.S. (Trondheim, Norway) in cooperation with the Norwegian University of Life Sciences and produced at Fôrtek (Aas, Norway). The feed mixture was pelleted through a 3.5 mm die and representative samples of each diet were collected and analyzed for chemical composition (Table 2). Feed production was described by (Cruz et al., 2019). Pellet durability index (%) was 96.6, 97.0, 97.7, and 98.1 for the control, CU10, CU20, and CU40 diets, respectively.

During the adaptation period, animals were offered a commercial weaner diet (Nutrimin A/S, Ans, Denmark), which was gradually replaced by 50, 75 and 100% of the respective experimental diets during the first three days. The animals were fed individually, twice daily at 0800 h and 1500 h. Feed amounts were adjusted daily to *ad libitum* based on the initially estimated feed intake of 2 to 4% of BW. Water was provided *ad libitum* via automatic drinkers. Individual feed leftovers were collected after each meal and recorded daily for calculating average daily feed intake. Average daily gain (ADG) and feed conversion ratio (FCR) were calculated from the individual BW and feed intake. A cumulative feed sample from each diet was collected for analysis of dry matter (DM), ash, starch, N, crude fat, neutral detergent fiber, gross energy and amino acids.

2.3. Experimental procedures

During the balance period and respiration experiments, the pigs were kept individually, in stainless steel metabolism cages (1.65 × 0.75 m) with devices for quantitative collection of feces, urine and feed residues which were collected quantitatively daily (between 0800 h and 1200 h) during the balance periods. Urine was collected in flasks containing 30 ml of 5% sulfuric acid solution. Citric acid (1% solution) was used to rinse the metabolic cages and was collected separately at the end of each collection. All samples of feces, urine, feed residues, and citric acid solutions were frozen at − 20 °C after the collections and thawed at the end of each period. Feces were individually homogenized, and urine, feed residues, and citric acid solutions were individually mixed. All samples were subsampled: approximately 20% of feces and 10% of urine were frozen at – 20 °C; feed residues and citric acid rinse solution were sampled representatively and frozen at – 20 °C. Feed samples of each diet were collected daily, pooled per period and frozen at – 20 °C.

The individual respiration measurements were performed in two

Table 1
Dietary composition and calculated content of nutrients and energy of experimental diets for young pigs with increasing levels of inactivated *Cyberlindnera jadinii* yeast.

Ingredients, g/kg	Diets[1]			
	Control	CU10	CU20	CU40
Wheat	624	616	608	593
Barley	100	100	100	100
Oats	50	50	50	50
Cyberlindnera jadinii[2]	0	36	73	146
Soybean meal[3]	80	65	50	19
Fish meal[4]	20	16	13	5
Potato protein concentrate[5]	38	30	23	9
Rapeseed meal[6]	20	16	12	5
Rapeseed oil	22	22	23	25
Sodium chloride	6	6	6	5
Monocalcium phosphate	13	14	14	16
Limestone	9	9	9	9
Iron (Fe)	0.4	0.4	0.4	0.4
Vitamin + trace-mineral premix[7]	4.8	4.9	4.9	5.0
L-Lysine	6.3	6.3	6.1	5.8
L-Methionine	2.1	2.3	2.5	3.0
L-Threonine	2.8	2.8	2.6	2.4
L-Valine	1.0	1.0	1.0	1.0
L-Tryptophan	0.9	0.9	0.9	1.0
Calculated content				
Net energy[8] (MJ/kg)	9.94	9.94	9.94	9.94
Crude protein g/kg	170	170	170	170
Digestible protein (SID) g/kg	140	140	140	140
Digestible Lys (SID) g/kg	12.0	12.0	12.0	12.0
SID Met-g/kg	4.6	4.7	4.8	4.9
SID Thr-g/kg	7.6	7.6	7.6	7.6
SID Val-g/kg	8.0	8.0	8.0	8.0
SID Try g/kg	2.6	2.6	2.6	2.6
Crude protein from *C. jadinii* (%%)	0.0	10.0	20.1	40.3

[1] Control diet; diet with 10% crude protein (CP) from *Cyberlindnera jadinii* (CU10); diet with 20% CP from *C. jadinii* (CU20); diet with 40% CP from *C. jadinii.* (CU40).

[2] Dried, inactivated: dry matter (DM) 970 g/kg, CP ($N \times 6.25$) 470 g/kg, crude fat 16 g/kg, ash 78 g/kg, gross energy 19.9 MJ/kg; essential amino acid content in g/16 g N: 24.4 Arg, 8.5 His, 21.6 Ile, 31.6 Leu, 30.6 Lys, 5.2 Met, 18.4 Phe, 25.6 Thr, 25.9 Val, 6.2 Trp.

[3] Non-GMO soybean meal, Denofa AS, Fredrikstad, Norway: DM 881 g/kg, CP 458 g/kg, crude fat 10 g/kg, ash 56 g/kg, neutral detergent fiber (NDF) 89 g/kg, gross energy 17.5 MJ/kg.

[4] Norsildmel AS, Egersund, Norway: DM 917 g/kg, CP 684 g/kg, crude fat 73 g/kg, ash 145 g/kg, NDF 5 g/kg, gross energy 19.4 MJ/kg.

[5] Cargill, Denmark: DM 914 g/kg, CP 725 g/kg, crude fat 30 g/kg, ash 20 g/kg, gross energy 21.8 MJ/kg.

[6] Expeller pressed rapeseed meal, Mestilla, UAB, Klaipeda, Lithuania: DM 889 g/kg, CP 350 g/kg, crude fat 88 g/kg, ash 59 g/kg, NDF 161 g/kg, gross energy 19.1 MJ/kg.

[7] Provided per kg of diet: 120 mg Fe ($FeSO_4$); 60 mg Mn (MnO); 120 mg Zn (ZnO); 26 mg Cu ($CuSO_4$); 0.60 mg I (Ca (IO_3)); < 0.3 mg Se; 8000 IU vitamin A; 45 mg dl-α-tocopheryl acetate; 105 mg ascorbic acid; 1500 IU cholecalciferol; 4.64 mg menadione; 3 mg thiamin; 5.63 mg riboflavin; 45 mg niacin; 15 mg pantothenic acid; 20 μg cyanocobalamin.

[8] Calculated based on Centraal Veevoederbureau (2005).

Table 2
Analyzed chemical composition of experimental pig diets with increasing levels of inactivated *Cyberlindnera jadinii* yeast.

Item, g/kg	Diets[1]			
	Control	CU10	CU20	CU40
Dry matter	877	914	881	887
Crude protein	183	180	174	176
Crude fat	39	48	48	49
Starch	443	448	455	458
Ash	48	51	49	50
Carbohydrates + lignin[2]	608	635	610	612
Neutral detergent fiber	97	96	96	85
Gross energy (MJ/kg)	16.5	16.8	17.3	16.7
Essential AA[3] (g /16 g N)				
Arg	9.3	9.1	8.8	8.7
His	3.7	3.6	3.5	3.4
Ile	7.1	6.8	6.8	6.6
Leu	12.5	12.1	11.7	11.2
Lys	13.1	13.0	12.8	12.3
Met	4.4	4.5	4.5	4.7
Phe	7.9	7.6	7.4	7.0
Thr	9.5	9.6	9.3	9.5
Val	9.5	9.3	9.2	9.1
Trp	2.8	2.9	2.9	2.8
Non-essential AA (g/16 g N)				
Ala	7.2	7.3	7.3	7.8
Asp	14.4	13.8	13.2	12.6
Gly	7.6	7.4	7.2	7.0
Glu	35.0	34.8	34.7	34.3
Cys	2.6	2.5	2.4	2.2
Tyr	3.1	3.3	3.1	3.1
Pro	11.9	11.7	11.6	10.9
Ser	8.5	8.6	8.3	8.4

[1] Control diet; diet with 10% crude protein (CP) from *Cyberlindnera jadinii* (CU10); diet with 20% CP from *C. jadinii* (CU20); diet with 40% CP from *C. jadinii* (CU40).

[2] Calculated as carbohydrates + lignin = dry matter - ash - CP - crude fat.

[3] Amino acids.

open-air-circuit respiration chambers with a volume of 3500 L. Construction and function of the respiration chambers have been described by Chwalibog et al. (2004). The airflow through the chambers and the concentrations of O_2, CO_2 and CH_4 in the out-going air from each chamber were recorded automatically every third minute. Gas recovery tests were performed for CO_2 and O_2 in both chambers with the following results: Chamber A CO_2 = 1.0968 and O_2 = 1.0757; Chamber B O_2 = 1.0016 and O_2 = 1.0333. The temperature was set at 20 °C, the relative humidity varied between 60 and 65% and 12 h light-dark cycles were operated. All pigs were continuously monitored by video surveillance during the respiration experiments.

At the end of each experimental period, the animals were euthanized by captive bolt pistol and exsanguination.

2.4. Analytical methods

Fecal samples were freeze-dried. Feces and diets were ground to pass a 1 mm sieve before chemical analysis. Feed residues were analyzed for DM content, and it was assumed that the chemical composition of the DM was equal to that in the DM of the feed. Wet fecal samples were analyzed for N and DM. Diets and freeze-dried fecal samples were analyzed for DM, ash, crude fat, and gross energy. Diets were additionally analyzed in triplicate for AA including tryptophan. Urine and citric acid rinse samples were analyzed for N content. The DM, N, ash, crude fat and AA were determined according to the methods described in European Commission (2009). The DM was measured by drying to constant weight at 103 °C. Ash was determined by incineration at 525 °C. The N content was determined using the Tecator-Kjeltec system 1030 (Tecator AB, Höganäs, Sweden). Fat content was determined by petroleum ether extraction in a Soxtec system 2043 (Foss, Hillerød, Denmark) after HCl hydrolysis. The gross energy in feed and feces was determined using an IKA Calorimeter system (IKA GmbH and Co. KG, Staufen, Germany), according to the method described by the International Organization for Standardization (1998). The diets were additionally analyzed for neutral detergent fiber as described by Mertens (2002), using the Ankom200 Fiber Analyzer (ANKOM Technology, Macedon, New York, USA). Amino acids were determined on a Biochrom 30 Amino Acid Analyzer (Biochrom Ltd, Cambridge, UK). Tryptophan was analyzed on a Dionex UltiMate 3000 HPLC system (Dionex Softron GmbH, Germering, Germany) with a Shimadzu RF-535 fluorescence detector (Shimadzu Corporation, Kyoto, Japan). Starch was hydrolyzed with α-amylase and amyl glucosidase-

enzymes to glucose, and glucose concentration was determined using a spectrophotometer (MaxMat PL II Multianalyzer, France) as described by McCleary et al. (1994).

2.5. Calculations

The CP content was calculated as $N \times 6.25$. Total carbohydrate and lignin (CHO + *L*) content in the diets and feces was calculated as CHO + L = DM − ash − CP − crude fat. The apparent total tract digestibility (ATTD) of nutrients and energy was calculated as ATTD (%) = [(nutrient intake − nutrient in feces) / nutrient intake] × 100 (Maynard and Loosli, 1969).

Nutrient intake was calculated based on feed intake (feed intake = amount of feed provided − feed residues) and the analyzed nutrient content of the feed. Nutrients in feces were calculated based on the total weight of the feces collected and the analyzed nutrient content in fecal samples.

Retained nitrogen (RN) was calculated as RN (g) = IN − UN − FN – NCA, where, IN is the ingested nitrogen, UN the urinary nitrogen, FN the fecal nitrogen, and NCA the concentration of nitrogen in the citric acid rinse solution. Digestible nitrogen (DN, g) was calculated as DN = IN − FN. Energy in urine (UE, kJ) was calculated as UE = 53.5 kJ/g × UN (g) (Chwalibog et al., 2004). Energy in methane (ECH_4, kJ) was calculated as $ECH_4 = 39.6\ kJ/l \times CH_4$ (l) (Brouwer, 1965). Metabolizable energy intake (MEi, kJ) was calculated as MEi = GEi – FE − UE − ECH_4, where GEi is the gross energy intake. Heat production (HE) was calculated based on the 22-h measurements of gas exchange and the mean UN according to Brouwer (1965) as HE (kJ) = 16.18 (kJ/l) × O_2 (l) + 5.02 (kJ/l) × CO_2 (l) − 2.17 (kJ/l) × CH_4 (l) − 5.99 kJ/g × UN (g), where 22-h volumes of O_2, CO_2, and CH_4 were extended to 24 h by multiplication with 24/22. Retained energy (RE, kJ) was calculated as RE = ME − HE. Energy retained as protein (RPE, kJ) was calculated as RPE = RN (g) × 6.25 × 23.86 (kJ/g) and energy retained as fat (RFE, kJ) as RFE = RE – RPE. The respiratory quotient (RQ) was determined as RQ = CO_2 production / O_2 consumption. The oxidation of protein, carbohydrate and fat were calculated according to Chwalibog et al. (1992) and validated for RQ values below and above one (Chwalibog and Thorbek, 1995).

2.6. Statistical Analysis

Statistical analysis of growth performance, ATTD, metabolism of N and energy were performed using the general linear model multivariate procedure of SPSS statistics software v25 (IBM Corporation, Armonk, New York, 2017). Fixed effects of diet ($n = 4$) and period ($n = 3$), were included in the statistical model for the growth performance, ATTD, and N and energy metabolism analyses. Interactions between diet and period were investigated and removed from the model when non-significant. Dietary treatment means were separated using the estimated marginal means (an equivalent of the least square means) option of the general linear model. Pearson's' correlation was used to investigate relationships between initial BW and the ATTD of nutrients. Effects were considered significant if $P < 0.05$. Linear, quadratic and cubic effects of the dietary treatments on the ATTD of nutrients, and the metabolism of N and energy were investigated using polynomial contrasts, but these were found to be non-significant ($P > 0.1$).

3. Results and discussion

3.1. General health and growth performance

Pigs generally remained healthy throughout the experiment, however, there were some occurrences of loose stools (fecal score > 2), but no watery diarrhea was observed (fecal score = 4). Means for DM of feces (%) during the balance period were 26.2, 27.8, 31.4 and 28.4 for pigs fed the control, CU10, CU20, and CU40 diets ($P = 0.112$). Fecal scores of the pigs were not affected by dietary treatment ($P > 0.1$). On the contrary, feeding piglets increasing levels of dietary *C. jadinii,* replacing up to 40% of the CP, induced a linear increase in fecal DM (Cruz et al., 2019), possibly explained by the positive effects of the mannooligosaccharides, β-glucans and nucleic acids present in the yeast-cells on the intestinal function of piglets (Kogan and Kocher, 2007; Mateo et al., 2004; Mateo and Stein, 2004).

Table 3
Growth performance of young pigs fed diets with increasing levels of inactivated *Cyberlindnera jadinii* yeast.[1]

Performance	Diets[2] Control	CU10	CU20	CU40	SEM[3]	*P*-value Diet
Initial BW	16.97	16.64	16.58	16.58	1.13	0.994
Final BW	25.27	24.56	23.75	23.93	1.43	0.873
Weight gain (g/day)						
Overall	593	566	511	525	34.1	0.338
Week1	428	472	351	352	43.1	0.163
Week 2	758	660	672	698	63.7	0.708
Feed intake (g/day)						
Overall	858	831	792	802	49.1	0.778
Week 1	533	542	472	481	43.0	0.578
Week 2	1169	1107	1195	1104	68.5	0.865
FCR[4]						
Overall	1.46	1.46	1.55	1.52	0.05	0.494
Week 1	1.64	1.29	1.58	1.42	0.25	0.754
Week 2	1.66	1.70	1.65	1.60	0.11	0.940

[1] Values presented as least square means ($n = 6$); BW, body weight (kg).
[2] Control diet; diet with 10% crude protein (CP) from *Cyberlindnera jadinii* (CU10); diet with 20% CP from *C. jadinii* (CU20); diet with 40% CP from *C. jadinii* (CU40).
[3] SEM, pooled standard error of the means.
[4] FCR, feed conversion ratio, calculated as feed intake (g) : BW gain (g).

In general, ADG, feed intake, and FCR of the pigs were not affected by dietary treatment ($P > 0.1$, Table 3). Nevertheless, there was an effect of period on those values ($P < 0.01$) which reflected differences in initial BW among periods ($P < 0.001$; Table 3), with the exception of FCR, that was not affected by period during the balance experiment ($P > 0.1$). The pigs in period 1 were heavier than the pigs in period 2 and 3 ($P < 0.01$), and pigs in period 2 were heavier than in period 3 ($P < 0.05$) but initial BW did not differ among dietary treatments ($P = 0.994$). Similar findings have previously been reported by Øverland et al. (2010) who concluded that bacterial protein meal (BPM) could replace 41% of the dietary CP for piglets without compromising growth performance, and more recently, Cruz et al. (2019) who found no overall changes in growth performance of piglets fed up to 40% CP from the same *C. jadinii*. However, hydrocarbon-grown *S. cerevisiae* has previously replaced 6 to 29% of CP in FM-based diets for pigs, resulting in a tendency for better ADG and FCR in the pigs fed yeast-containing diets compared with the pigs fed FM control diets (Barber et al., 1971).

3.2. Apparent total tract digestibility (ATTD)

The ATTD of DM, organic matter (OM), N, ash, crude fat, carbohydrates and energy in young pigs fed the control diet and diets with different levels of *C. jadinii* (Table 4), was not affected by dietary treatment ($P > 0.1$). There was an effect of period on the ATTD of organic matter ($P = 0.020$), crude fat ($P = 0.013$), carbohydrate + lignin fraction ($P = 0.003$) and energy ($P = 0.036$) and a tendency for an effect of period on the ATTD of DM ($P = 0.053$), whereas the ATTD of N and ash were unaffected ($P > 0.1$). Period effects were likely related to the individual variation in initial BW.

Contrarily to this experiment, Cruz et al. (2019) found that the ATTD of CP and ash increased in the same pig diets with 40% CP from *C. jadinii* compared to the control diets. The fact that no differences regarding ATTD of N were found among diets in this study may be

Table 4
Effects of increasing dietary levels of inactivated *Cyberlindnera jadinii* yeast on the apparent total tract digestibility (ATTD) of nutrients and energy in young pigs.[1]

ATTD,%%	Diets[2] Control	CU10	CU20	CU40	SEM[3]	*P*-value Diet
Dry matter	87.2	86.9	87.7	87.6	0.5	0.611
Organic matter	88.5	88.3	89.1	89.0	0.4	0.538
Nitrogen	81.7	80.4	82.5	81.7	1.0	0.525
Ash	63.0	62.3	65.1	64.2	2.1	0.789
Crude fat	79.1	81.7	81.0	82.5	1.0	0.160
Carbohydrates + lignin	91.2	91.1	91.6	91.6	0.3	0.560
Energy	86.3	86.2	87.2	87.0	0.5	0.447

[1] Values are presented as least square means ($n = 6$).
[2] Control diet; diet with 10% crude protein (CP) from *Cyberlindnera jadinii* (CU10); diet with 20% CP from *C. jadinii* (CU20); diet with 40% CP from *C. jadinii* (CU40).
[3] SEM, pooled standard error of the means.

related to the lower initial BW and younger age of the pigs in the previous study. Alternatively, differences between these studies, may be related to the use the marker method by Cruz et al. (2019) compared with the total collection method used in the present experiment. The ATTD of N in pigs fed 6 to 29% of total CP from hydrocarbon-grown *S. cerevisiae* was not affected (Barber et al., 1971), which agreed with our results. As in the present experiment, the ATTD of N was also similar between pig diets where the sole protein source was BPM (88% *Methylococcus capsulatus*), and control diets based on FM (Skrede et al., 1998). Contrarily, the ATTD of N in pig diets seemed to decrease in diets with 35 to 51% BPM compared with a SBM-based control diet (Hellwing et al., 2007). It is noteworthy that the ATTD of N increases with N intake when the intake of CP is low, as a result of a relatively lower loss of endogenous N through feces. As protein digestibility varies with protein intake (Noblet et al., 2004), comparisons of ATTD between studies may be difficult (Eggum, 1973; Fan et al., 1994).

The ATTD of energy was similar among diets in the study by Hellwing et al. (2007), which agrees with our findings. The ATTD of fat gradually increased in pig diets with 18 to 51% CP from BPM (Hellwing et al., 2007) compared to the SBM-based control diet, whereas no such differences were observed in our experiment.

The ATTD of DM, OM, crude fat, carbohydrate fraction and energy increased with initial BW (Pearsons' $r = 0.57, 0.60, 0.58, 0.58, 0.60$; $P < 0.005$), whereas the ATTD of N and ash were unaffected ($P > 0.1$). These positive correlations support evidence that the ATTD of nutrients increases with BW in young pigs, which, can be associated with age differences in these pigs. This is further supported by an increasing trend in the activities of pancreatic trypsin, chymotrypsin, and amylase with increasing age of the pigs from d 35 to 56 observed by Jensen et al. (1997). The apparent digestibility of crude fiber and N in pigs increases with age (from 60 to 150 days), due to changes in the microbiota populations of the gut over time (Niu et al., 2015). Differences in the ATTD of nutrients between this study when pigs were 63 to 72 days old and the previous when pigs were on average 53 days old (Cruz et al., 2019) are likely to be explained by the younger age of the pigs at the time-point for sample collections. However, ATTD measured with yttrium oxide was similar compared with total collection measured in minks (Vhile et al., 2007) and it was previously demonstrated that ATTD is highly correlated between pigs and minks fed BPM (Skrede et al., 1998). Thus, it is suggested that the differences in ATTD observed between the present study and that by Cruz et al. (2019) are more likely due to differences in age rather than the method. In similarity to our study, the ATTD of nutrients in growing-pig diets with 17.5 to 52.5% of CP from BPM gradually replacing SBM, was not affected by the dietary treatments (Hellwing et al., 2007). In broiler chickens, the ATTD of fat was found to generally increase with age (Tancharoenrat et al., 2013), which agrees with our findings in pigs.

Table 5
Effects of increasing dietary levels of inactivated yeast *Cyberlindnera jadinii* on the nitrogen metabolism in young pigs.[1]

Item, g/kg$^{0.6}$	Diets[2] Control	CU10	CU20	CU40	SEM[3]	*P*-value Diet
Ingested nitrogen	5.46	5.08	5.09	5.37	0.23	0.540
Digested nitrogen	4.46	4.09	4.20	4.39	0.21	0.572
Excreted nitrogen	1.78	1.82	1.76	1.80	0.11	0.979
Fecal nitrogen	0.997	0.987	0.893	0.985	0.06	0.550
Urinary nitrogen	0.758	0.790	0.837	0.795	0.09	0.934
UN:FN[4]	0.777	0.808	0.950	0.818	0.10	0.619
Retained nitrogen	3.705	3.307	3.362	3.592	0.19	0.405
RN:IN[5]	0.677	0.652	0.660	0.668	0.02	0.716
RN:DN[6]	0.830	0.808	0.800	0.818	0.02	0.674

[1] Values are presented as least square means, ($n = 6$).
[2] Control diet; diet with 10% crude protein (CP) from *Cyberlindnera jadinii* (CU10); diet with 20% CP from *C. jadinii* (CU20); diet with 40% CP from *C. jadinii* (CU40).
[3] SEM, pooled standard error of the means.
[4] UN, urinary nitrogen; FN, fecal nitrogen.
[5] RN, retained nitrogen; IN, ingested nitrogen.
[6] DN, digested nitrogen.

The estimate of ATTD of carbohydrates + lignin was based on the calculated values for carbohydrate content, calculated from the analytical values for dry matter, ash, CP and crude fat in the diets and feces. This leads to accumulation of all analytical errors on the carbohydrate value (Neil, 1978), reducing the accuracy of that value, compared to those of the other nutrients here evaluated.

3.3. Nitrogen metabolism

Nitrogen metabolism in young pigs fed the experimental diets (Table 5) did not differ among dietary treatments or among periods ($P > 0.1$). The RN of the pigs in our experiment was correlated with initial BW (Pearson's $r = 0.81$; $P < 0.01$), which agrees with the study conducted by Barber et al. (1971). This relationship can be explained by an intensive growth in piglets after weaning, often accompanied by increasing N retention until the maximum capacity for N retention is reached (Tauson et al., 1998). It would be relevant to investigate the maximum N retention capacity for older cross-bred pigs, in further studies.

Retained N was similar in growing pigs fed diets with 6 to 29% of CP from hydrocarbon-grown *S. cerevisiae* yeast compared to pigs fed FM-based control diets (Barber et al., 1971), and was similar among pigs fed diets with up to 52.5% of CP from BPM, (Hellwing et al., 2007) and pigs fed control diets, which supports our results. However, RN was generally higher in the pigs in our study compared with the pigs fed BPM (Hellwing et al., 2007), which concurs with a comparably lower UN, and FN in the pigs in our study.

Yeast cells contain more non-protein N in the form of nucleic acids than most conventional protein sources, which can influence N digestibility and thus N metabolism (Skrede et al., 1998). This may reflect the slightly lower RN in the pigs fed the yeast-containing diets in our study and in the pigs fed BPM (Hellwing et al., 2007) compared with pigs fed control diets, indicating an increase in nucleic acids not directly available for protein synthesis in the diets, as a consequence of adding incremental levels of these microbial ingredients. The nucleic acid content of the yeast was not measured in our study but it has been previously reported that ribonucleic and deoxyribonucleic acids comprise approximately 7 to 9% of the composition of *C. jadinii* (Castro et al., 1971; Maul et al., 1970) equivalent to approximately 10% of the N in *C. jadinii*, while they comprise < 0.1% of the composition of other major protein sources (Mateo et al., 2004; Mateo and Stein, 2004) and 1% of the total N in FM (Barber et al., 1971).

Pigs fed diets with 18.7 to 56.0% of the CP from RSM (Pérez de

Table 6
Effects of increasing levels of inactivated *Cyberlindnera jadinii* yeast on the energy metabolism and substrate oxidation in young pigs.[1]

Item, kJ/kg$^{0.6}$	Diets[2]				SEM[3]	*P*-value
	Control	CU10	CU20	CU40		Diet
Digestible energy	2646	2623	2651	2798	122.1	0.736
Metabolizable energy	2602	2576	2604	2749	120.2	0.737
Heat production	1317	1399	1297	1272	73.0	0.645
Retained energy	1285	1177	1306	1477	121.7	0.398
RE:ME[4],%%	49	46	50	53	3.0	0.482
Energy retained in protein	550	487	497	532	27.4	0.352
Energy retained in fat	736	691	809	945	102.4	0.345
O_2 consumed (l/kg$^{0.60}$)	60.17	63.63	59.52	58.03	3.46	0.706
CO_2 produced ((l/kg$^{0.60}$)	69.35	74.53	67.72	67.28	3.74	0.515
CH_4 produced (l/kg$^{0.60}$)	0.09	0.09	0.05	0.09	0.03	0.689
Respiratory quotient	1.16	1.17	1.14	1.16	0.03	0.927

[1] Values are presented as least square means ($n = 6$).
[2] Control diet; diet with 10% crude protein (CP) from *Cyberlindnera jadinii* (CU10); diet with 20% CP from *C. jadinii* (CU20); diet with 40% CP from *C. jadinii* (CU40).
[3] SEM, pooled standard error of the means.
[4] RE, retained energy; ME, metabolizable energy.

Nanclares et al., 2019), ingested and digested less N, and excreted more N than the pigs in our experiment, which explains the higher RN in our experiment compared with the previous. The ratio RN : DN is a measure of the efficiency of utilizing DN for retention, and this was higher (80 to 83 vs. 64%) for the pigs in our study than those fed diets with 17.5 to 52.5% CP from BPM (Hellwing et al., 2007). Ratios RN : IN and RN : DN were also generally higher in pigs in our experiment compared with the pigs fed RSM-based diets (Pérez de Nanclares et al., 2019) resulting from higher RN in the present experiment. The higher RN : DN in the present experiment, as compared to the previous studies (Hellwing et al., 2007; Pérez de Nanclares et al., 2019) might be explained by the use of intact pigs, which have higher capacity for N retention than castrated pigs (Tauson et al., 1998). Additionally, rubber mats with openings were used in the metabolism cages in this experiment, to improve the comfort of the pigs, and this may have contributed to some loss of nitrogenous material compared with previous studies without mats.

3.4. Energy metabolism and substrate oxidation

Energy metabolism, measured by the consumption of O_2, and the production of CO_2 and of CH_4 (Table 6) did not differ among dietary treatments ($P > 0.1$). Period tended to affect digestible energy ($P = 0.065$) and ME ($P = 0.064$) intakes, which can be explained by an effect of initial BW as discussed above. The digestible energy, ME, HE and RE and energy retained as protein did not differ between pigs fed the control and pigs fed yeast-containing diets, agreeing with the findings of Hellwing et al. (2007), despite the slightly higher energy retained as protein in the present study. The digestible energy and ME were, however, lower for pigs fed diets with 18.7 to 56.0% of CP from RSM (Pérez de Nanclares et al., 2019) compared with the pigs in our study, which is likely associated with the higher content of fiber in barley and RSM, compared with wheat, and *C. jadinii*. Additionally, those pigs produced higher amounts of methane and CO_2 (Pérez de Nanclares et al., 2019) compared to the pigs fed yeast-based diets in our study. The lower methane production in the present experiment compared with the previous experiment by Pérez de Nanclares et al. (2019) might be explained by an increase in the passage of undigested nutrients to the hindgut and a higher fermentation rate by intestinal microflora, due to the higher fiber content in the barley and RSM-based diets.

Mean oxidized protein (7 to 8% of HE) and mean oxidized carbohydrates (92 to 93% of HE) were similar among dietary treatments ($P > 0.1$). Net oxidation of fat was zero because all pigs had RQ values > 1, indicating *de novo* lipogenesis from dietary carbohydrates (Chwalibog and Thorbek, 2000). Thus, in previous studies with zero net oxidation of fat, HE was mainly made up by carbohydrate oxidation (85%) while protein oxidation made up the remaining (15%) (Chwalibog et al., 1998). In our experiment carbohydrate oxidation was higher and protein oxidation was lower than the values from Chwalibog et al. (1998). The differences in substrate oxidation between experiments may also be caused by differences in collection procedures where perforated rubber mats in the metabolism cages may have caused losses on nitrogenous material in this study. Few studies have been performed to evaluate the metabolism of protein and energy in pigs fed diets containing microbial protein thus, further assessments with *C. jadinii* as a protein source for pigs are encouraged.

4. Conclusions

Replacing up to 40% of CP from SBM, FM, RSM, and potato protein concentrate with inactivated *Cyberlindnera jadinii* yeast CP in diets for young pigs had no adverse effects on N and energy metabolism or on feed intake, growth rate, general animal health and performance, compared with the control diet. The ATTD of dietary nutrients and energy was not affected by dietary treatment but was affected by the initial BW of the piglets. Altogether, the results suggest that partially replacing conventional protein sources with inactivated *C. jadinii* yeast in diets for young pigs is possible without compromising energy and protein metabolism.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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