1 ***Candida utilis* yeast as a protein source for weaned piglets: Effects on growth**
2 **performance and digestive function**

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15 **Abstract**

16 Yeast such as inactivated *Candida utilis* produced from lignocellulosic biomass from
17 underutilized wood co-products as a second-generation sugar source is a potentially
18 sustainable protein feed ingredient in diets for piglets. This study aimed to evaluate the effects
19 of *C. utilis* added to diets for weaned piglets on growth performance and digestive function
20 when replacing main protein sources. Forty-eight piglets weaned at 30 days of age, with a
21 mean starting weight of 11.06 ± 0.84 kg were fed one of four dietary treatments for 28 days: a
22 conventional control diet with soybean meal, fishmeal, rapeseed meal, and potato protein or
23 one of three experimental diets containing 10, 20 or 40% crude protein (CP) from yeast
24 (CU10, CU20, and CU40, respectively). Adding yeast to diets did not affect growth
25 performance compared with the control. The diet with 40% CP from *C. utilis* had higher
26 apparent total tract digestibility (ATTD) of CP compared with the control ($P = 0.034$) and
27 higher ATTD of ash ($P < 0.001$) compared with the control. The ATTD of neutral detergent
28 fiber decreased in the CU40 diet compared with the control ($P = 0.006$). The apparent ileal
29 digestibility (AID) of ash increased ($P = 0.001$) in the CU40 diet compared with the control,
30 while the AID of CP and amino acids was unaffected. Villi-height increased in jejunum ($P =$
31 0.007) and ileum ($P = 0.047$), and villus-height: crypt-depth ratio increased ($P = 0.001$) in
32 jejunum of piglets fed the CU40 diet compared with the control. Fecal dry matter increased
33 linearly with increasing levels of *C. utilis* in the diets at day 7 after weaning ($P = 0.001$) and
34 was higher for the CU40 group compared with the control group at day 21 after weaning ($P =$
35 0.027). Trypsin activity and messenger RNA expression of nine genes encoding for nutrient
36 transporters in the jejunum did not differ among diets. Collectively, the results indicated that
37 *C. utilis* can replace 40% of CP from the main protein sources traditionally used in diets for
38 weaned piglets while maintaining growth and improving digestive function.

39

40 **Keywords** *Candida utilis*, yeast, growth performance, digestive function, weaned piglet

41

42 ## 1. Introduction

43 The livestock industry in Norway is challenged by a high dependence on imported feed
44 ingredients such as soybean meal because of a limited supply of locally produced protein
45 resources (de Visser et al., 2014; Øverland and Skrede, 2017). To improve national self-
46 sufficiency of food, it is necessary to develop alternative methods to acquire protein
47 resources. Recent advances in biorefining technology using lignocellulosic biomass as a
48 source of second-generation sugars enable the production of locally-produced protein sources
49 such as yeast (Øverland and Skrede, 2017). Yeast cells and their derivatives are known for
50 their β-glucan, mannooligosaccharide and nucleic acid contents, to induce immunostimulant
51 effects in piglets (Hahn et al., 2006; White et al., 2002) and reduce post-weaning diarrhea.
52 Dietary yeast for pigs has shown beneficial effects on health when used in small amounts
53 (White et al., 2002), but limited information exists on the nutritional value of yeast in larger
54 amounts as a protein source in piglet diets. However, Spark et al. (2005) demonstrated that
55 growth performance in piglets improved when 20 to 60% of soybean meal was replaced by 6
56 to 17% dietary yeast, due to a reduction in the content of anti-nutritional factors in the diet.
57 *Candida utilis* yeast (more recently classified as *Cyberlindnera jadinii*) grown on
58 lignocellulosic biomass has not been previously tested in diets for pigs. The aim of this study
59 was therefore to determine the effects of this locally-produced *C. utilis* as a protein source on
60 the growth performance and digestive function of weaned piglets.

61

62 ## 2. Materials and Methods

63 ### *2.1. Animals and facilities*

64 All the animals were handled in accordance with the applicable laws and regulations
65 controlling experiments with live animals in Norway (the Animal Welfare Act of 28 of
66 December 2009 and the local legislation derived from the directive 2010/63 EU of the
67 European Parliament and Council of 22 September 2010 on the protection of animals used for
68 scientific purposes). The experiment was approved by the Norwegian Food Safety Authority
69 (identification number: 11314). The experiment was performed at the Center for Livestock
70 Production, Norwegian University of Life Sciences, Aas, Norway, from February to March of
71 2017 and lasted for twenty-eight days. Twelve sows (Norwegian Landrace × Yorkshire)
72 inseminated with boar semen (Duroc) provided the piglets for this experiment. At
73 approximately thirty days of age (29.6 ± 1.05 standard deviations [SD]), and an average initial
74 body weight of 11.06 kg ± 0.84 SD, twenty-five surgically castrated-male piglets, and twenty-
75 three intact-female piglets, were equally distributed by litter, gender, and weight and
76 randomly allotted to four dietary treatments, with twelve replicates per treatment. Pigs within
77 the same pen received the same diet. At the stipulated feeding times, each pig was separated
78 from the others in an individual feeding stall for 30 min to measure individual feed intake.
79 Thus, each pig constituted an experimental unit. All piglets were healthy at the start of the
80 experiment. Each group of four piglets was kept in a concrete-floored, partially slatted pen of
81 3.35 × 2.25 m with individual feeding areas of 0.37 × 1.35 m each. A rubber mat of
82 approximately 90 × 100 cm was used as a replacement for other bedding materials, to
83 minimize interference with the measurements of digestibility and gastrointestinal health
84 effects of the diets. Heating lamps were installed over the rubber mats to provide comfortable
85 resting areas and the pens were equipped with activity enrichment toys. The room temperature
86 was kept on average at 19.05°C ± 1.74 SD, with 8 hours of light and 16-hour darkness cycles.
87 During the hours of darkness, a night light was used.

88 ***2.2. Yeast single-cell protein***

89 *Candida utilis* biomass (LYCC 7549; Lallemand Yeast Culture Collection) was produced by
90 Lallemand Inc, Salutaguse, Estonia. Second generation sugars were obtained from
91 lignocellulosic biomass of Norway spruce trees (*Picea abies*) by using the Borregaard
92 Advanced Lignin process at Borregaard AS, Sarpsborg Norway (Patent "Lignocellulosic
93 biomass conversion by sulfite pretreatment"; EP2376642B1 EP Grant). The C5 and C6 sugars
94 were used in the growth media for the yeast, as described by Øverland and Skrede (2017) and
95 Sharma et al. (2018).

96 ***2.3. Diets and feeding***

97 The diets were formulated in collaboration between Felleskjøpet Fôrutvikling A.S. and the
98 Norwegian university of life sciences and produced at the Center for Feed Technology
99 (Fôrtek), Norwegian university of life sciences, Aas, Norway. Feed ingredients were ground
100 through a 3 mm die using a hammer mill (Roskamp, California, USA). Fine materials were
101 transported into an automated dosing and batching system (Abel Company, Wisconsin, USA).
102 All ingredients were mixed with a twin shaft paddle mixer (Dinnissen, Netherlands). The
103 mash was conditioned at 74 to 76 °C and pelleted (Twin Pass, Muench, Germany). The
104 finished pellets were cylindrical 3 × 10 mm and the pellet temperature varied from 82.4 to
105 93.4 °C. The dietary treatments consisted of one control diet and three experimental diets. The
106 experimental diets consisted of a gradual replacement of the main sources of CP, soybean
107 meal, potato protein concentrate, fishmeal and rapeseed meal with drum dried and inactivated
108 *C. utilis* corresponding to 10, 20 or 40% of the total CP content. Thus, the diets were coded in
109 order as control, CU10 (10% CP from yeast), CU20 (20% CP from yeast) and CU40 (40% CP
110 from yeast). The chemical composition of soybean meal, potato protein concentrate, fishmeal,
111 rapeseed meal and *C. utilis* is shown under Table 1. The diets were formulated to be
112 isonitrogenous and isoenergetic based on the analyzed chemical content of the ingredients
113 (Table 1). A replica of each diet was produced in separate batches and added the inert marker

114 Yttrium (III) oxide (Y_2O_3) was added at 0.01% to these replicas. The analyzed chemical
115 composition of the diets is shown in Table 2. Piglets were fed three times per day during the
116 first 14 days and two times per day during the remaining period. Feed was provided *ad*
117 *libitum* during restrictive time periods and the amounts of feed were adjusted weekly, based
118 on estimated feed intake of 3 to 5% of the live body weight. Water was accessible *ad libitum*
119 via automatic drinkers. Diets containing Y_2O_3 were provided from day 18 of the experiment
120 for the determination of apparent total tract digestibility (ATTD) and apparent ileal
121 digestibility (AID). Individual feed leftovers were collected after each meal and recorded
122 weekly for calculating average daily feed intake (ADFI). Individual live body weight was
123 recorded weekly for calculating average daily gain (ADG) and feed conversion ratio (FCR). A
124 cumulative feed sample from each diet was collected for analysis of dry matter, ash, starch,
125 CP, crude fat, neutral detergent fiber, gross energy and amino acids (AA).

126 ***2.4. Fecal score and dry matter***

127 Fecal score was registered daily for 28 days and for each pen (n = 12) on a scale from 1 to 4,
128 according to consistency (1 = dry and hard; 2 = normal; 3 = pasty, with loss of normal shape;
129 4 = watery) to assess the presence of diarrhea (fecal score ≥3) (Pedersen and Toft, 2011). In
130 addition, fecal samples were collected weekly by pen for determination of dry matter.

131 ***2.5. Sample collection***

132 On the last day of the experiment, the piglets were fed 2.5 hours before euthanasia, to ensure
133 the presence of enough intestinal content for sample collection. All animals were euthanized
134 with a captive bolt pistol and exsanguination. Intestinal content and tissue samples were
135 collected from the aboral portion of jejunum and ileum. Jejunal content was collected for
136 analysis of trypsin activity. Intestinal segments, heart, lung, liver, kidney, and other organs
137 with gross lesions were collected for morphological studies. Samples of jejunum were

138 collected for quantification of nutrient transporter expression. Total liver weight was
139 recorded, and liver index was calculated as: liver index = liver weight (kg) / live body weight
140 (kg).

141 ***2.6. Digestibility***

142 For determination of the ATTD, individual fecal samples were collected from the floor after
143 defecation, consecutively from experiment days 21 to 25. The fecal samples were pooled,
144 freeze-dried, ground at 0.5 and 1 mm and homogenized before analyses. Immediately after
145 slaughter, intestinal contents were collected from the last two meters of the ileum and jejunum
146 from each animal, for determination of AID. The intestinal content and fecal samples were
147 analyzed for Y_2O_3 concentrations and nutritional content based on the methods described by
148 Austreng et al. (2000). Apparent digestibility of nutrients was calculated as described by
149 Maynard and Loosli (1969).

150 ***2.7. Morphology and intestinal morphometry***

151 To evaluate the general health status of the pigs, all abdominal and thoracic organs and the
152 remaining carcass, were evaluated for gross lesions while sampling. Gross lesions were
153 recorded, and additional samples were taken for histology and/or microbiology when
154 indicated. In addition, histomorphology was performed on tissues from the heart, lung, liver,
155 and kidney from all pigs. Heart, lung, liver, kidney and intestinal tissue samples for histology
156 were collected within 20 min of euthanasia and fixed in 10% formalin. The gut tissues from
157 the 48 individuals were sectioned along the mesenteric attachment and the serosal surface was
158 placed on a piece of cardboard prior to formalin fixation. After 48 hours of fixation, the
159 tissues were routinely processed, embedded in paraffin and cut in 4 µm sections. Sections
160 were deparaffinized in xylene and rehydrated in graded alcohol before routine staining with
161 hematoxylin and eosin. Formalin-fixed, paraffin-embedded tissue sections were also stained

162 with high iron diamine and alcian blue (HID-AB). Digital images of the intestinal sections
163 were captured using an Axiocam 105 color digital sight camera configured with a Zeiss
164 Lab.A1 microscope. Morphometric measurements were performed using the software ImageJ
165 1.51k (National Institutes of Health, USA). For villus height (VH) and crypt depth (CD)
166 measurements and VH:CD calculations, villi, and crypts were chosen from the stem of
167 mucosal folds not containing Peyer's patches. The longest villi in proximity to well-oriented
168 crypts were selected and micrographs were captured at 10× magnification, while the longest
169 crypts in the same micrographs were selected for measurements of the CD. VH was measured
170 by drawing a segmented line through the villus center extending from the tip to the villus-
171 crypt-junction. CD was measured from the villus-crypt junction to the basement membrane of
172 the deepest portion of the crypt, adjacent to the *tunica muscularis mucosae*. Between three
173 and six villi and crypts were measured in each intestinal segment from each piglet. VH:CD
174 for each intestinal segment was calculated using the mean VH and mean CD of the villus-
175 crypt complexes.

176 ### *2.8. Enzyme Activity*

177 Approximately 100 mg of contents from the jejunum were collected and snap-frozen at − 80
178 °C. The samples were thawed, homogenized and centrifuged at 21,100 × g for 5 min at 4 °C.
179 The supernatant was analyzed for trypsin activity and total protein concentration using
180 commercial kits according to manufacturer's instructions (Trypsin Activity Assay kit, Abcam,
181 Cambridge, UK and Bio-Rad Protein Assay, Bio-Rad, California, USA).

182 ### *2.9. Gene expression of intestinal nutrient transporters*

183 #### *2.9.1. RNA extraction*

184 Total RNA from jejunum was extracted from 7 pigs fed the control diet and 8 pigs fed the
185 CU40 diet, using TRIzol TM protocol (Invitrogen) followed by RNeasy Plus Mini protocol

186 (Qiagen). After the first washing step, on-column DNAse treatment was performed using the
187 PureLink DNase kit (Invitrogen). RNA purity and quality were measured using NanoDrop
188 8000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA) and Agilent 2100
189 Bioanalyzer (Agilent Technologies, Waldbronn, Germany). Only high quality (RNA integrity
190 number ≥ 7) samples were used for quantitative real-time PCR (polymerase chain reaction)
191 analysis.

192 ***2.9.2. cDNA synthesis and quantitative real-time PCR***

193 The gene expression for the following nutrient transporters was measured: *glucose*
194 *transporter 2, glucose transporter 4, sodium-glucose cotransporter 1, monocarboxylate*
195 *transporter 1, fatty acid binding protein 1, fatty acid binding protein 2, fatty acid binding*
196 *protein 6, peptide transporter 1* and *intestinal alkaline phosphatase.* The primers used for
197 quantitative real-time PCR are shown in Table 3. Complementary DNA (cDNA) synthesis
198 was performed using the AffinityScript QPCR cDNA Synthesis kit (Agilent Technologies).
199 The quantitative real-time PCR was performed in a total volume of 20 µL using 10 µL
200 LightCycler 480 SYBR Green I Master, 2 µL primers, 3 µL Milli-Q water and 5 µL cDNA
201 diluted 1:50. The specificity of PCR amplification was confirmed with melting curve analysis.
202 The PCR conditions were: 95 °C for 10 min, 95 °C for 10 seconds, 60 to 64°C for 10 seconds
203 depending on the primers, 72 °C for 10 seconds, in a total of 40 cycles. Samples were
204 analyzed using the LightCycler 480 System (Roche Diagnostics, Mannheim, Germany).
205 *Glyceraldehyde-3-phosphate dehydrogenase* and *β-actin* were tested as reference genes, but
206 only *β-actin* showed stable expression across samples and treatments and was used in the
207 analysis. All reactions were performed in duplicate and the transcriptional levels of selected
208 genes were quantified relative to the expression of *β-actin* using a mean − ΔΔCt value.

209 ***2.10. Chemical analysis***

210 The chemical analyses of ingredients, feed, ileal and fecal samples were performed by the
211 LabTek group, Norwegian university of life sciences, Norway. Ingredients and diets were
212 ground at 1 mm and 0.5 mm for chemical analysis of main nutrient content. The diets were
213 analyzed in triplicate for dry matter, ash, starch, CP, crude fat, neutral detergent fiber, energy
214 content and AA including tryptophan. Fecal samples and ileal content were freeze-dried,
215 homogenized and analyzed in duplicate for dry matter, ash, starch, and CP. Fecal samples
216 were additionally analyzed for crude fat, neutral detergent fiber, and gross energy content.
217 Ileal samples were also analyzed for AA and tryptophan. Dry matter, ash, CP (Kjeldahl-
218 nitrogen × 6.25) and AA were determined according to the methods described in the
219 European Commission Regulation (EC) No 152/2009. AA were analyzed using the Biochrom
220 30 Amino Acid Analyzer (Biochrom Ltd., Cambridge, UK). Tryptophan was analyzed on a
221 Dionex UltiMate 3000 HPLC system (Dionex Softron GmbH, Germering, Germany) with a
222 Shimadzu RF-535 fluorescence detector (Shimadzu Corporation, Kyoto, Japan). Neutral
223 detergent fiber was analyzed as described by Mertens, (2002) using the Ankom200 Fiber
224 Analyzer (ANKOM Technology, Macedon, New York, USA). Gross energy content was
225 determined by a PARR 1281 Adiabatic Bomb Calorimeter (Parr Instruments, Moline, Illinois,
226 USA) (method International Organization for Standardization, 1998). Crude fat was
227 determined using Accelerated Solvent Extraction (ASE350, Dionex Corporation, Sunnyvale,
228 California, USA). Feed samples were extracted with 70% petroleum-ether and 30% acetone at
229 125 °C. Ileal and fecal samples were extracted with 80% petroleum and 20% acetone at 125
230 °C. Starch was hydrolyzed with α-amylase and amyl glucosidase-enzymes to glucose, and
231 glucose concentration was determined using a spectrophotometer (MaxMat PL II
232 Multianalyzer, France) as described by McCleary et al. (1994). Yttrium (Y-89) concentrations
233 in samples were determined by inductively coupled plasma mass spectroscopy using an
234 Agilent 8800 Triple Quadrupole ICP-MS/MS (Agilent Technologies Inc., Santa Clara, USA)

235 in oxygen reaction mode, at the Department of Environmental Sciences, Norwegian
236 university of life sciences. The samples were digested in concentrated nitric acid (HNO_3) in
237 an UltraCLAVE III (Milestone, Sorisole, Italy) at 260 °C for 15 min, and diluted with
238 deionized water before analysis.

239 ***2.11. Statistical analysis***

240 For statistical analyses of performance, digestibility and fecal score, the general linear model
241 procedure with the least square means method in SAS software 9.4 (SAS Inst. Inc., Cary,
242 North Carolina, USA) was used with the STDERR PDIFF options and adjusted for TUKEY
243 to investigate differences ($P < 0.05$) between the dietary treatment groups. P-values between
244 0.05 and 0.1 were considered tendencies. The CONTRAST statement was used to investigate
245 differences between the control and the yeast diets. Linear correlations between amounts of
246 CP from yeast in the experimental diets and the growth performance parameters were
247 investigated using the linear regression procedure. For the statistical analyses of VH, CD and
248 VH:CD, Graph pad prism 7.0 (2016 GraphPad Software, Inc., California, USA) was used to
249 investigate associations between each diet, VH, CD, VH:CD, ADG and FCR by performing
250 unpaired t-tests and determining Pearson's' correlation coefficients. When a parameter was
251 measured for each animal such as ADFI, ADG, FCR, digestibility, liver index, and enzyme
252 activity, the piglet was considered the statistical unit and data were analyzed according to the
253 model $Y_{ijkmn} = \mu + \alpha_i + \beta_j + \tau_k + \eta_{m'}(\beta_j) + \varepsilon_{ijkmn}$, where Y_{ijkmn} is the dependent variable
254 (animal), μ represents the overall sample mean, α_i the dietary treatment effect (i = 1, 2, 3, 4),
255 β_j the litter effect (1,2,..12), τ_k the effect of sex (k = female, male), $\eta_{m'}(\beta_j)$ the random effect
256 of pen (m = 1, 2,.. 12) when given same dietary treatment and ε_{ijkmn} the residual error. The
257 model was reviewed for each group of parameters; when no effect on nutrient digestibility
258 was shown, the variable sex was excluded from the model. Litter and pen showed no
259 significant effect on the liver index and were therefore removed from the statistical model for

260 this analysis. In statistical analyses of fecal dry matter and fecal score, pen constituted the
261 experimental unit. Diets were included as explanatory effects according to the following
262 model $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$. For the gene expression analysis of nutrient transporters in jejunum, a
263 two-sample *t*-test was performed to investigate differences between the control and yeast
264 groups. Based on chemical composition, it was suspected that there was an error in the batch
265 of the diet CU20 that included the digestibility marker, thus the results from this diet were
266 removed from the statistical analysis. This included ADG, FCR, fecal dry matter between
267 days 14 and 28, AID, ATTD, trypsin activity, and intestinal morphometry.

268 ## 3. Results

269 ### *3.1. General health and growth performance*

270 There was no mortality during the experimental period. During the first three days of the
271 experiment, fecal score was 1 for all pens (n = 12). There were some occurrences of diarrhea
272 (fecal score ≥ 3) during the experimental period, especially during the second week. The
273 average fecal score for the overall period were 2.3, 2.1, 2.1 and 2.1 for the control, CU10,
274 CU20, and CU40 diets respectively (P = 0.563). During the second week of the experiment,
275 the average fecal score were 2.7, 2.8, 2.6 and 2.4 for the control, CU10, CU20, and CU40
276 diets respectively (P = 0.550). During the fourth week of the experiment, the average fecal
277 score for the pigs fed the control diet was higher compared to the pigs fed the yeast-
278 containing diets (2.4 for the control group vs. 2.1 and 1.8 for the CU10 and CU40 groups
279 respectively; P = 0.044). Fecal dry matter (%) increased linearly with increasing levels of
280 dietary yeast (P = 0.001) at day 7 of the experimental period (18.3 for the control group vs.
281 22.5, 24.1, 27.7 for CU10, CU20 and CU40, groups respectively) and was higher for pigs fed
282 the CU40 diet compared with the pigs fed the control diet (30.4 vs. 27.1; P = 0.027) at day 21.
283 The liver index increased in piglets fed the CU40 diet compared with piglets fed the control

284 diet (3.20 vs. 2.89; P = 0.022). The yeast diet CU40 tended to improve FCR during the last
285 two weeks of the experiment (P = 0.099). In general, no statistical differences among dietary
286 treatments were observed in ADG, ADFI, and FCR (Table 4).

287 ***3.2. Digestibility and enzymatic activity***

288 Results for the apparent digestibility of AA and other nutrients are shown in Table 5 and
289 Table 6, respectively. There were no differences in AID of dry matter, CP or most AA among
290 diets, however, AID of methionine and alanine tended to be highest in the CU40 diet (P =
291 0.064, P = 0.084). AID of ash was higher in the group fed the CU40 diet (P = 0.001). AID of
292 starch tended (P = 0.096) to be lower in the CU10 diet. ATTD of CP of the CU40 diet was
293 higher than the control (P = 0.034). ATTD of neutral detergent fiber was lower for the CU40
294 diet compared with the control (P = 0.006). ATTD of crude fat was higher for the CU40 diet
295 compared with CU10 (P = 0.035). ATTD of ash increased in the CU10 and CU40 diets
296 relative to the control diet ($P < 0.001$). ATTD of phosphorus increased in the CU40 diet
297 compared with the control ($P < 0.001$). No differences in trypsin activity among dietary
298 treatments were observed (P = 0.812). Numerical means for trypsin activity for the control,
299 CU10 and CU40 diets were 2.20, 2.45 and 2.43 U/mg protein respectively.

300 ***3.3. Messenger RNA (mRNA) expression of nutrient transporters in jejunum***

301 Results for expression of nutrient transporters are shown in Table 7. The expression of the
302 selected genes was not affected by dietary treatments. Although statistically not different
303 between treatments, the gene with the highest expression in CU40 fed pigs compared with the
304 control was the *intestinal alkaline phosphatase* encoding a digestive brush-border enzyme,
305 while the *fatty acid binding protein 6,* regulating uptake, transport, and metabolism of fatty
306 acids, had the lowest expression in pigs fed the CU40 diet compared with the pigs fed the
307 control diets.

308 ***3.4. Morphology***

309 Macroscopic evaluation of the pigs revealed a mild to moderate hyperkeratosis of the
310 cutaneous mucosa of the ventricle in all animals and was independent of the dietary
311 treatments. Sixteen of 48 pigs (33.3%) had peritonitis, 13 of these pigs (27.1%) had chronic
312 peritonitis comprising mild fibrous thickening of peritoneum over cecum and colon, and three
313 pigs (6.3%) presented signs of active inflammation with hyperemia and sparse amounts of
314 small fibrin flakes. A navel abscess was observed in one animal and a small abscess was
315 observed on the thigh of another animal. Two pigs (4.2%) presented mild chronic thickening
316 of the mitral valve. Renal cysts were observed in two animals (4.2%). *Staphylococcus aureus*
317 and *Streptococcus dysgalactiae* were isolated from a bacterial culture of the navel abscess.
318 Histomorphological evaluation of the lungs demonstrated very mild to mild, multifocal to
319 diffuse, subacute interstitial pneumonia in forty-five of the piglets (93.8%). Seven of twelve
320 animals in the control group and one of twelve animals in the CU40 group had very mild
321 multifocal hepatitis, with infiltrations of few aggregates of neutrophils, lymphocytes, and
322 macrophages multifocally in the liver parenchyma. No specific findings were observed in the
323 myocardium and kidney samples.

324 ***3.5. Intestinal morphometry***

325 Results for intestinal morphometry are presented in Table 8. VH, CD, and VH:CD were
326 compared between the control and CU40 diets. Pigs fed the CU40 diet had longer VH in the
327 jejunum (P = 0.007) and ileum (P = 0.047) compared with pigs fed the control diet. VH:CD in
328 jejunum increased in pigs fed the CU40 diet compared with the control diet (P = 0.001). The
329 ileal CD measurements differed between these two feeding groups. Ileal crypts were deeper in
330 the control group than in the group receiving the CU40 diet (P = 0.018). Ileal VH was

331 negatively correlated with FCR (Figure 1) in pigs fed the CU40 diet (r = − 0.61, P = 0.035).
332 No correlation between ileal VH and FCR was found in the control group.

333 ## 4. Discussion

334 The reliance on imported protein-rich ingredients in Norway has attracted increased interest in
335 the research and development of competitive locally-produced protein sources. The present
336 study demonstrated the potential of *C. utilis* yeast as an alternative protein source to soybean
337 meal, fishmeal, rapeseed meal, and potato protein concentrate in pelleted diets for weaned
338 piglets. Weaning is a critical life-stage for piglets because they are exposed to several stress
339 factors (social, nutritional and immunological) that frequently result in diarrhea and reduced
340 growth performance. This study showed that it is possible to replace 40% of CP by using *C.*
341 *utilis* in commercial-like diets for weaned piglets while maintaining ADG and FCR. Similar
342 results in growth performance have been reported in other studies using yeast as a protein
343 source for pigs or Atlantic salmon (*Salmo salar*), while others have reported improved growth
344 performance. Improvement in growth performance may occur because of increased ADFI
345 (Lezcano et al., 2013) due to enhanced palatability of the feed (Ati et al., 2009). Hu et al.
346 (2014) observed improved FCR of piglets fed diets containing 8% CP derived from baker's
347 yeast (AB Yestex), but they did not observe differences in ADFI among piglets fed the
348 control compared with those fed the yeast-based diets. Øverland et al. (2013) reported no
349 difference in ADFI, growth rate or FCR between Atlantic salmon fed a fishmeal-based control
350 diet and a test diet containing 28.3% *C. utilis*, replacing 40% of the CP. However, at high
351 levels, yeast may reduce the palatability of the diet, as shown in a study with diets containing
352 up to 75% dried brewers' yeast fed to rainbow trout (*Oncorhynchus mykiss*) and consequently
353 lower feed intake (Rumsey et al., 1991a). In a study with broiler chickens, the partial
354 replacement of soybean meal with 10% vinasse yeast resulted in a higher weight gain
355 compared with the control diet, while at the higher inclusion levels of 20 or 30%, the addition

356 of yeast to diets led to reduced weight gain and increased FCR (Lezcano et al., 2013). Unlike
357 the findings in the present study, Lezcano and co-workers (2013) observed that feed intake
358 increased by including 10, 20 and 30% of *C. utilis* yeast in diets compared with the control.

359 Yeast cell-walls are rich in mannooligosaccharides, which can bind glycoprotein receptors in
360 pathogenic bacteria and limit their attachment to the intestinal mucosa (Refstie et al., 2010).
361 This mechanism promotes the passage of pathogenic bacteria through the intestine without
362 causing infection and reduces the consumption of dietary protein by pathogenic bacteria,
363 which could otherwise be available for digestion and absorption (Ewing and Cole, 1994;
364 Kogan and Kocher, 2007). In the present study, providing 40% of yeast CP resulted in higher
365 ATTD of CP of pigs fed the CU40 diets compared with the control. The improved ATTD of
366 CP could be a result of the observed increased VH and VH:CD, which indicates an increased
367 intestinal absorption area in the piglets fed the CU40 diet compared with the piglets fed the
368 control diets. In our study, the dietary treatments did not induce differences in the expression
369 of the nine selected genes involved in nutrient sensing and transportation, however, an
370 increase in VH:CD can be associated with higher expression of genes coding for nutrient
371 transporters. Heim et al. (2015) found a connection between increased VH:CD in ileum and
372 higher expression of nutrient transporter *sodium-glucose cotransporter 1* in piglets, implying
373 improved absorption ability. In this study, no differences in the expression of *sodium-glucose*
374 *cotransporter 1* were observed in the jejunum.

375 The activity of digestive enzymes may be affected by yeast, either directly or indirectly,
376 although differences for trypsin activity were not observed in our study. Live yeast can
377 provide digestive enzymes, favoring efficient digestion of complex carbohydrates which
378 could potentially also exert a positive effect on protein, fat and mineral digestibility (Øverland
379 and Skrede, 2017). However, the enzymes provided by *C. utilis* in this experiment were
380 likely inactivated during the downstream processing of this ingredient. The increased ash

381 digestibility in the diets containing *C. utilis* compared with the control diet could be due to a
382 high bioavailability of the minerals in the yeast. In accordance with our results, Kim et al.
383 (2000) reported higher phosphorus digestibility in boars fed diets containing brewers' yeast.
384 Improved mineral digestibility may have been related to increased VH (Heidarieh et al.,
385 2013). The lower digestibility of the neutral detergent fiber in diets with yeast, especially in
386 the CU40 diet compared with the control, could be due to the cell wall of the yeast, which
387 constitutes on average 29% of dried yeast cells (Nguyen et al., 1998), and possesses low
388 digestibility (Rumsey et al.,1991b).

389 Growth performance relies on healthy intestinal tissue capable of absorbing nutrients in the
390 amounts necessary to meet the nutritional requirements for maintenance and growth. VH, CD,
391 and VH:CD measurements in jejunum and ileum can be used as indicators of general
392 intestinal function and health. Our results showed longer VH in yeast-fed piglets compared
393 with the control group and inversely the CD was shorter in the pigs fed the yeast-based diet
394 CU40 compared to the control group. Heidarieh et al. (2013) discussed the relationship
395 between improved FCR and increased VH in pigs and Shen et al. (2009) concluded that
396 dietary yeast culture supplementation at 0.5% had a positive effect on growth performance of
397 nursery pigs by improving jejunal VH and VH:CD. The longer VH in piglets fed yeast-based
398 diets in our study could be suggestive of a reduced contact between pathogenic bacteria and
399 the intestinal wall in the yeast-fed group, and thus less damage to the villi, compared with the
400 pigs fed the control diet. Deeper intestinal crypts in the control pigs fed the control diet could
401 be the result of increased cell proliferation to repair damaged villi tissue, caused by the
402 adherence of intestinal pathogens to the intestinal mucosa in these pigs. Due to the adsorbing
403 properties of *C. utilis*, this might have been prevented in the yeast fed pigs (Ewing and Cole,
404 1994; Kogan and Kocher, 2007). Alternatively, increased VH and consequently VH:CD could
405 be related to the modulating effect of yeast in gut immune responses (Shen et al., 2009). Shen

406 and co-workers demonstrated a comparable effect of 0.5% of yeast culture supplementation
407 and antibiotic growth promoters on the growth performance of nursery pigs, which provides
408 evidence for the yeast's ability to counteract pathology and promote health. We thus speculate
409 that the mechanisms involved in the intestinal health effects of dietary *C. utilis* are mainly
410 immune-and-microbial modulation as previously described in other studies with pigs (Hahn,
411 et al., 2006; Shen et al., 2009) and fish (Siwicki et al., 1994).

412 Pathogens and inflammatory processes in the intestine may interfere with the efficiency of
413 nutrient absorption. Repartitioning of energy from growth to inflammation and immune-
414 stimulation processes may, in turn, lead to reduced animal performance (Fox et al., 2005;
415 Grammes et al., 2013). Mannooligosaccharides, β-glucans and nucleic acids in *C. utilis* may
416 contribute to improve intestinal health (Refstie et al., 2010), and reduce inflammation
417 (Grammes et al., 2013), which could explain the increased intestinal absorption surface
418 indicated by our study. This explanation is further supported by the correlation between FCR
419 and the ileal mucosal-surface area, found in our study where the FCR decreased with
420 increasing ileal VH in the CU40 group.

421 Post-weaning diarrhea is often associated with a decrease in productive performance in piglets
422 and can be assessed in herds by subjective fecal score or determination of fecal dry matter.
423 These methods provide indications though they are not standard methods to determine
424 diarrhea (Pedersen et al., 2011). The linear increase in fecal dry matter at day 7 with
425 increasing levels of yeast in the diets suggest an improvement of the intestinal health status.

426 The CP content in *C. utilis* attracted our interest as a potential and competitive protein source
427 when compared to soybean meal. *C. utilis* used in the present study contained 48% CP (on dry
428 matter basis) and had a high content of threonine, but a low content of methionine, cysteine,
429 and arginine when compared with other commonly used protein sources in Norwegian pig

430 diets. However, the low content of the mentioned essential AA was considered when
431 formulating the diets by adjusting to a similar AA level by addition of crystalline AA. The
432 protein level in *C. utilis* was on average similar to those reported by Martin et al. (1993)
433 (52.0%) and Olvera-Novoa et al. (2002) (46.1%), while Øverland et al. (2013) reported higher
434 CP level (59.8%). *C. utilis* is also rich in nucleic acids, which are known to have positive
435 effects on intestinal development and regeneration (Mateo et al., 2004). Endogenous
436 nucleotides depleted during stressful periods, such as weaning, may be restocked by nucleic
437 acid-rich compounds such as yeast, which in turn may have a role in preventing losses in
438 growth performance, common for pigs during this life-stage (Mateo et al., 2004). In our
439 experiment, *C. utilis* may have to some extent contributed to maintain the nucleic acid balance
440 in the intestine and promote better intestinal health, also expressed by the increased VH.
441 These results agree with the documented positive effects of *C. utilis* (Grammes et al., 2013)
442 and *S. cerevisiae* (Refstie et al., 2010) on intestinal health.

443 **5. Conclusions**

444 Replacing up to 40% of CP from the traditional protein sources with CP from the yeast *C.*
445 *utilis* in piglet diets had no effect on feed intake and growth rate of the piglets while the
446 ATTD of CP was improved. Adding *C. utilis* to diets also resulted in longer intestinal villi,
447 increased VH:CD and improved fecal consistency in the piglets. These findings suggest that
448 *C. utilis* can replace 40% of CP from the main protein sources traditionally used in Norway
449 while maintaining growth performance and improving digestive function.

450 ***Conflict of interest statement***

451 The authors declare that they have no conflict of interest.

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457

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572

573 **Table 1**

574 Dietary composition of the experimental diets.

Item	Diet[1]			
	Control	CU10	CU20	CU40
Formulation, g/kg, as is				
Wheat	624	616	608	593
Barley	100	100	100	100
Oats	50	50	50	50
Yeast meal[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]	4.2	4.2	4.2	4.2
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	170	170	170	170
Crude protein from *Candida utilis* (%)	0.0	10.0	20.1	40.3

575 [1] Control diet (Control); diet with 10% crude protein (CP) from *Candida utilis* (CU10); diet with 20% CP from
576 *C. utilis* (CU20); diet with 40% CP from *C. utilis.* (CU40).
577 [2] Dried inactivated *C. utilis*: dry matter (DM) 970 g/kg, CP (N × 6.25) 470 g/kg, crude fat 16 g/kg, ash 78 g/kg,
578 gross energy 19.9 MJ/kg; essential amino acid content in g/16g N: 24.4 Arg, 8.5 His, 21.6 Ile, 31.6 Leu, 30.6
579 Lys, 5.2 Met, 18.4 Phe, 25.6 Thr, 25.9 Val, 6.2 Trp.
580 [3] Non-GMO soybean meal, Denofa AS, Fredrikstad, Norway: DM 881 g/kg, CP 458 g/kg, crude fat 10 g/kg, ash
581 56 g/kg, neutral detergent fiber (NDF) 89 g/kg, gross energy 17.5 MJ/kg.
582 [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,
583 gross energy 19.4 MJ/kg.
584 [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.
585 [6] Expeller pressed rapeseed meal, Mestilla, UAB, Klaipeda, Lithuania: DM 889 g/kg, CP 350 g/kg, crude fat 88
586 g/kg, ash 59 g/kg, NDF 161 g/kg, gross energy 19.1 MJ/kg.
587 [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
588 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
589 cholecalciferol; 4.64 mg menadione; 3 mg thiamin; 5.63 mg riboflavin; 45 mg niacin; 15 mg pantothenic acid;
590 20 μg cyanocobalamin.
591 [8] Calculated based on Central Veevoederbureau (2005).
592

593 **Table 2**

594 Analyzed chemical composition of experimental diets.

Item, g/kg	Diet[1]			
	Control	CU10	CU20	CU40
Dry matter	882	878	885	890
Crude protein	177	169	170	174
Crude fat	36	40	45	43
Starch	443	448	455	458
Ash	54	48	50	52
Neutral detergent fiber	97	96	96	85
Gross energy (MJ/kg)	16	17	17	17
Essential AA[2] (g /16g 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/16g 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

595 [1] Control diet (Control); diet with 10% crude protein from *Candida utilis* (CU10); diet with 20% crude protein

596 from *C. utilis* (CU20); diet with 40% crude protein from *C. utilis* (CU40).

597 [2] Amino acids.

598

599 **Table 3**

600 Primers used for real-time quantitative PCR.

Primer Name	Abbreviation	Sequence (5´-3´) [a]	Product size (bp)	Accession Number
Fatty acid binding protein 1	*FABP1*	F-CTTCTCCGGCAAATACCAAG R-CCCGGTAGTGATGGTCAACT	160	NM_001004046.2
Fatty acid binding protein 2	*FABP2*	F- TAACTACAGCCTCGCAGACG R- GACCATTTCATCCCCGATAA	139	NM_001031780.1
Fatty acid binding protein 6	*FABP6*	F- GTGCGACATAGAGACCATCG R- TAGTTGGGGCTGTTCACCA	87	NM_214215.2
Peptide transporter 1	*PEPT1*	F- AATTGTGTCGTTGTCCAT R-AAGTCTGTGACTCATTG	78	NM_214347.1
Glucose transporter type 2	*GLUT2*	F-GTTCATGGTGGCCGAGTT R-ATTGCGGGTCCAGTTGC	82	NM_001097417.1
Glucose transporter type 4	*GLUT4*	F- TAAGACAAGATGCCGTCGGG R-GAGAAGACGGCGAGGACAAG	98	NM_001128433.1
Sodium-glucose cotransporter 1	*SGLT1*	F-TGTCTTCCTCATGGTGCCAA R-AGGAGGGTCTCAGGCCAAA	149	NM_001164021.1
Monocarboxylate transporter 1	*MCT1*	F-GGTGGAGGTCCTATCAGCAG R-AAGCAGCCGCCAAAATCAT	74	NM_001128445.1
Alkaline phosphatase, intestinal	*ALPI*	F-AGGAACCCAGAGGACCATTC R-CACAGTGGCTGAGGGACTTAGG	83	XM_003133729.4
β-actin	*ACTB*	F-CCAGGTCATCACCATCGG R-CCGTGTTGGCGTAGAGGT	158	XM_021086047.1
Glyceraldehyde 3-phosphate dehydrogenase	*GAPDH*	F- ACACTCACTCTTCTACCTTTG R- CAAATTCATTGTCGTACCAG	90	NM_001206359.1

601 [a]F, forward, R, reverse.

602

603 **Table 4**

604 Effects of dietary *Candida utilis* on the growth performance of weaned piglets.[1]

Item	n	Diet[2]				SEM[3]	P-value
		Control	CU10	CU20	CU40		
Initial BW[4], kg	48	11.08	11.06	11.13	11.00	0.12	0.986
Final BW[4], kg	48	21.07	20.46	19.62	20.64	0.25	0.203
Average daily gain, g							
Day 0-14	47	181	175	208	195	13.09	0.297
Day 14-28	36	486	482	-	516	17.89	0.338
Overall period	36	334	328	-	352	12.15	0.335
Average daily feed intake, g							
Day 0-14	47	275	263	294	278	10.89	0.233
Day 14-28	36	639	651	-	651	16.44	0.834
Overall period	36	457	457	-	467	11.67	0.790
FCR[5], g/g							
Day 0-14	47	1.59	1.53	1.49	1.43	0.074	0.460
Day 14-28	36	1.32	1.38	-	1.27	0.033	0.099
Overall period	36	1.38	1.41	-	1.33	0.030	0.228

605 [1]Results are given as least square means. Values with a distance from the grand mean larger than 3 times the
606 interquartile range were excluded from the analysis.
607 [2]Control diet (Control); diet with 10% crude protein from *Candida utilis* (CU10); diet with 20% crude protein
608 from *C. utilis* (CU20); diet with 40% crude protein from *C. utilis* (CU40).
609 [3]SEM, pooled standard error of the means.
610 [4]BW, live body weight.
611 [5]FCR, feed conversion ratio, calculated as feed: gain.
612
613

614 **Table 5**

615 Effects of feeding diets with up to 40% crude protein from *Candida utilis* on the apparent ileal
616 digestibility of amino acids in weaned piglets.[1]

617

Item	n	Diet[2]			SEM[3]	P-value
		Control	CU10	CU40		
Apparent ileal digestibility, %						
Arg	35	84.0	84.2	85.9	0.87	0.234
His	36	81.9	81.1	83.3	0.98	0.307
Ile	35	80.7	80.0	80.2	1.07	0.868
Leu	35	83.1	82.8	83.5	0.92	0.863
Lys	36	87.3	87.1	88.8	0.75	0.255
Met	36	90.9	90.6	92.3	0.52	0.064
Phe	36	82.9	81.7	82.9	0.95	0.567
Thr	36	81.8	80.4	78.3	1.17	0.131
Trp	36	83.7	84.0	84.3	0.72	0.830
Val	36	81.4	80.1	80.9	1.09	0.719
Ala	36	74.9	74.4	78.5	1.31	0.084
Asp	36	75.9	75.0	78.5	1.21	0.127
Cys	36	72.8	72.3	72.7	1.51	0.974
Glu	36	86.0	86.5	87.6	0.93	0.463
Gly	36	60.3	56.0	61.8	4.50	0.647
Pro	35	74.5	77.1	74.3	2.42	0.679
Ser	36	79.1	77.8	77.5	1.24	0.621
Tyr	36	71.7	70.8	71.9	1.35	0.829

618 [1] Results are shown as least square means. Values with a distance from the grand mean larger than 3 interquartile
619 range were excluded from the analysis.
620 [2] Control diet (Control); diet with 10% crude protein from *Candida utilis* (CU10); diet with 40% crude protein
621 from *C. utilis* (CU40).
622 [3] SEM, standard error of the mean.
623

624 **Table 6**

625 Effects of dietary *Candida utilis* on the apparent ileal digestibility and apparent total tract
626 digestibility of nutrients in weaned piglets.[1]

627

Item	n	Diet[2]				
		Control	CU10	CU40	SEM[3]	P-value
Apparent ileal digestibility, %						
Dry matter	36	73.1	74.1	73.6	1.33	0.865
Crude protein (N × 6.25)	36	76.7	76.1	78.8	1.40	0.389
Starch	34	98.2	98.0	98.6	0.20	0.096
Ash	36	39.9[a]	45.3[a]	54.9[b]	2.29	0.001
Apparent total tract digestibility, %						
Dry matter	35	83.2	83.5	83.9	0.28	0.264
Starch	35	99.7	99.7	99.7	0.02	0.545
Crude protein (N × 6.25)	35	78.3[a]	79.8[ab]	80.0[b]	0.44	0.034
Crude fat	35	71.0[ab]	69.4[a]	74.4[b]	1.25	0.035
Neutral detergent fiber	35	36.1[a]	33.2[ab]	25.5[b]	2.10	0.006
Ash	35	55.0[a]	59.1[b]	59.6[b]	0.66	< 0.001
Ca	34	61.0	63.0	63.6	-	0.219
P	35	51.0[a]	54.1[a]	58.0[b]	0.92	< 0.001
Gross energy	35	82.4	83.0	83.2	0.28	0.164

628 [1]Results are given as least square means. Values with a distance from the grand mean larger than 3 times the
629 interquartile range were excluded from the analysis.

630 [2]Control diet (Control); diet with 10% crude protein from *Candida utilis* (CU10); diet with 40% crude protein
631 from *C. utilis* (CU40).

632 [3]SEM, pooled standard error of the means.

633 [a-b] Means within a row with different superscripts differ ($P<0.05$).

634

635

636 **Table 7**

637 Jejunal expression of genes involved in the regulation of nutrient uptake in piglets fed diets
638 with 40% crude protein from *Candida utilis* compared with the control diet as measured by
639 quantitative real-time PCR.[1]

Item	Gene[2]								
	FABP1	*FABP2*	*FABP6*	*ALPI*	*SGLT1*	*PEPT1*	*MCT1*	*GLUT2*	*GLUT4*
Mean, − ΔΔCt	0.23	− 0.23	− 1.00	0.37	− 0.08	− 0.40	0.30	− 0.01	0.24
P-value	0.43	0.52	0.62	0.23	0.81	0.21	0.15	0.97	0.57

640 [1]Results are presented as mean − ΔΔCt (n = 8) relative to the control group (n = 7). Transcriptional levels of
641 selected genes were normalized to *β-actin*.

642 [2]FABP1, Fatty acid binding protein 1; FABP2, Fatty acid binding protein 2; FABP6, Fatty acid binding protein
643 6; ALPI, Alkaline phosphatase, intestinal; SGLT1, Sodium-glucose cotransporter 1; PEPT1, Peptide transporter
644 1; MCT1, Monocarboxylate transporter 1; GLUT2, Glucose transporter type 2; GLUT4, Glucose transporter
645 type 4.

646

647 **Table 8**

648 Effects of dietary *Candida utilis* on the intestinal morphometry of weaned piglets.[1]

Item	Diets[2]				P-value
	Control	SEM[3]	CU40	SEM[3]	
Jejunum					
VH (µm)	430.8	20.86	520.6	21.60	0.007
CD (µm)	356.2	12.15	342.4	13.55	0.455
VH:CD	1.22	0.06	1.53	0.06	0.001
Ileum					
VH (µm)	409.9	20.43	414.9	10.86	0.047
CD (µm)	314.3	9.29	286.5	5.69	0.018
VH:CD	1.31	0.07	1.45	0.04	0.089

649 [1] VH, villus height; CD, crypt depth. Results are given as means of three to six observations
650 per gut segment per piglet, n = 24.

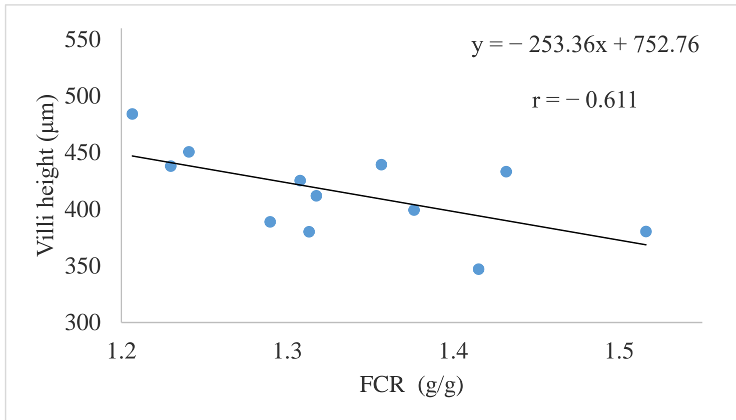
651 [2] Control diet (Control); diet with 40% crude protein from *Candida utilis* (CU40).

652 [3] SEM, standard error of the mean.

653

654 **Figure 1**

655 Relationship between average ileal villi height and overall feed conversion ratio (FCR), in
656 piglets fed diets with 40% crude protein from *Candida utilis*.[1]



657

658 [1]FCR, Feed conversion ratio, g feed intake per g weight gain; (n = 12). 95% confidence
659 interval − 0.8776 to − 0.0584; $P < 0.05$.