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Dietary inclusion of methanotrophic microbial cell-derived protein in the early postweaning period sustains growth performance and intestinal health of weaner piglets

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ABSTRACT

The global demand for sustainably produced protein feeds for animal production is increasing. Methanotrophic bacteria grow on methane and convert it into microbial cell protein (**MCP**) that has been shown to have high nutritive value for growing pigs. The present aimed to investigate how increasing amounts of MCP in diets fed during the first 15 days after weaning affect the growth performance of piglets from weaning until day 43 postweaning. Furthermore, the effect of MCP on intestinal morphology and histopathology was assessed on day 15 after weaning. During seven consecutive weeks, approximately 480 piglets were recruited for the experiment per batch. The piglets were divided into four groups and housed in eight double pens with 60 piglets per pen. The piglets were fed one of four experimental diets with 0, 3, 6, or 10% of MCP included at the expense of fishmeal and subsequently potato protein for the first 15 days postweaning. Thereafter, all pigs were fed commercial weaner diets in two phases (days 16–30 and days 31–43) until day 43 postweaning. All diets were without medicinal zinc. Feed intake and growth were registered on double pen level during all three phases. On day 15 after weaning, 10 piglets per treatment were randomly selected, autopsied, and sampled for intestinal morphology and histopathology. Daily gain during the first 15 days postweaning tended ($P = 0.09$) to be affected by the inclusion of MCP in the weaning diet being lowest in the group fed 10% MCP. Treatment did not affect daily feed intake; however, Feed Conversion Ratio (**FCR**) was significantly affected ($P = 0.003$) showing the highest FCR in piglets fed 10% MCP. Growth performance was not affected by the experimental treatment during the following phases. In the small intestine, villous height tended ($P = 0.09$) to show a quadratic response to level of MCP in the diet with the longest villi observed after feeding 6% MCP. Dietary treatment did not affect crypt depth. The villous height to crypt depth (**VC**) ratio showed a quadratic response to increased dietary inclusion of MCP ($P = 0.02$) with piglets fed 6% MCP having the highest VC ratio. In conclusion, this study demonstrated that MCP could constitute 6% of diets as-fed (22% of total CP), at the expense of fishmeal and potato protein, for newly weaned piglets without negative effects on growth rates and FCR. The inclusion of MCP in diets for newly weaned piglets could be part of improving the sustainability of pig production.

Implications

The demand for increased sustainability in pig production has emphasised the need for alternative protein sources for feeding livestock. Microbial cell protein can be grown on methane with minimal dependence on soil and water which reduces the competition for arable land for human food production. This study shows that microbial cell protein may constitute 6% of diets as-fed (22% of total CP) for newly weaned piglets without negative effects on growth rates and intestinal health and hence could be part of improving the sustainability of pig production.

Introduction

The global demand for sustainably produced protein feeds for humans is predicted to increase in the future in line with increased

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population growth. This has encouraged efforts to search for new sustainably produced protein ingredients for feeding livestock. Production of feed protein should be associated with high security of supply and high nutritional value (Matassa et al., 2016; Bratosin et al., 2021) and preferably without a competition for arable land for human food production (Matassa et al., 2016).

Single-cell protein, i.e. protein derived from bacteria, fungi (including yeast) or algae, is of increasing interest due to their rapid growth rates and the possible use of inexpensive waste materials from food and feed industries as well as forestry and agricultural residues as substrates (Ritala et al., 2017). A microbial culture consisting of *Methylococcus capsulatus* (Bath) (NCIMB strain 11132), *Alcaligenes acidovorans* (NCIMB strain 13287, former taxonomic name *Ralstonia* sp.), *Bacillus brevis* (NCIMB strain 13288, former taxonomic name *Aneurinibacillus danicus*) and *Bacillus firmus* (NCIMB strain 13289, former taxonomic name *Brevibacillus* sp.) can convert methane into microbial cell protein (**MCP**) and is able to grow with minimum dependence on soil and water (Øverland et al., 2010). MCP contains approximately 70% crude protein (Anupama and Ravindra, 2000) and has an amino acid composition very similar to that of fishmeal and soybean meal (Øverland et al., 2010), except for lower lysine and higher tryptophan concentrations (Rønn et al., 2022). The nutritive value of MCP for growing pigs has been investigated in several studies (Skrede et al., 1998; Øverland et al., 2004; Hellwing et al., 2007); however, production methods have been refined and studies on the use of MCP for weaning piglets are scarce (Øverland et al., 2001; Zhang et al., 2013). A recent study showed that MCP has superior content of standardised ileal digestible essential amino acids relative to digestible lysine as compared to fishmeal (Rønn et al., 2022).

A high content of nucleic acids in foods can make MCP unsuitable for human consumption (Kuhad et al., 1997), as the breakdown of purine bases increases uric acid concentration in plasma, which can cause gout and kidney stones (Wiederkehr and Moe, 2011). However, in contrast to humans, pigs are able to convert uric acid to allantoin which is easily excreted in urine (Maiuolo et al., 2016). In fact, nucleotides are important building blocks particularly during rapid cell growth and division, as in the intestinal mucosa and immune cells of young piglets. In pigs, nucleotides have therefore been proposed as alternative feed additives during the postweaning period to exert their beneficial effects on intestinal development, immune function, and nutrient metabolism (Sauer et al., 2011; Valini et al., 2021). High dietary contents of nucleic acids may therefore not represent a health problem for pigs, but quite contrary contribute to alleviate health problems in the immediate postweaning period.

The aim of this experiment was to investigate, how increasing amounts of MCP in diets fed during the first 15 days after weaning would affect the growth performance of piglets from weaning until day 43 postweaning (~30 kg bodyweight). In the experimental diets, MCP substituted currently used high-quality and expensive protein sources, namely fishmeal (**FM**) and potato protein **(PP)**. Furthermore, to evaluate the effect of dietary inclusion rate of MCP on intestinal morphological development and immune-related traits, a subset of piglets was autopsied at day 15 postweaning.

Material and methods

Experimental facility

The present experiment was conducted at the experimental station TestGris (Skjoldborg test station, Herning, Denmark) managed as a commercial farm. The farm has approximately 1 100 sows and nine weaner piglet sections managed by the all-in/all-out principle. In this facility, batches of piglets are weaned weekly and inserted into an empty, clean weaner section with space for 750 piglets divided into 24 single pens (2.4 m × 4.3 m with one-third slatted floor and two-thirds solid concrete floor in the resting area). Every two pens share dry feed dispensers for automatic feeding *ad libitum* and water supply, placed in the wall partitioning the double pen. The feed dispenser was placed in the area with a slatted floor. As enrichment, each pen was equipped with a wooden beam in a vertical rack (a schematic figure of the pens can be seen in Supplementary Fig. S1). The diets were supplied by the dispenser into the feeding trays, whenever requested by a weight sensor. When delivered to the individual feed dispensers, the amount of diet dropped into the feeder trays was recorded by weight. Hence, feed administration registrations from a section were available per double pen, whereas group weights of piglets were available per single pen.

Experimental design, animals, and feeding

A total of 3 375 piglets (Landrace/Yorkshire × Duroc) were used in this dose–response feeding experiment. The piglets were weaned from sows in their 2nd to 4th parity with litter sizes standardised 2 days postpartum to 16 piglets. The piglets were vaccinated against *E. coli* (Coliprotec F4/F18, Elanco Denmark ApS, Ballerup, Denmark) 1 week prior to weaning. During each of seven consecutive weeks, approximately 480 piglets were recruited for the experiment at weaning. The average weaning age was 24–25 days (minimum 21 days, maximum 30 days). The piglets were assigned to one of four size classes at weaning (small, small-medium, medium-large, large) upon visual inspection, excluding runts. The groups (size classes) were then randomly inserted into eight double pens with on average 31.9 ± 3.2 piglets in each single pen. Piglets in the two single pens forming a double pen had the same size class and were assigned to the same treatment (two replications per treatment per batch; in total 14 replications per treatment), and the size classes were evenly distributed to all treatments across the experiment.

Feeding and recordings

The piglets had prior to weaning been offered a pelleted (diameter 2.8 mm) prestarter (Danish140, Net energy (MJ/kg feed as-is (Feed Units/kg (FU/kg))): 9.30 (1.26), CP: 20.24%, digestible CP: 176.55 g/kg, digestible lysine: 15.12 g/kg, Danish Agro, Karise, Denmark) from four days of age by an automated feeding system (Uni-Feeder, Unitron A/S, Kolding, Denmark), and had access to water *ad libitum* via drinking nipples.

The piglets were assigned to one of four experimental diets from the day of weaning until 15 days after weaning: a control diet with FM and refined PP as protein feed sources (0% MCP in feed as-fed) and three diets with increasing inclusion rates of MCP (3, 6 or 10% in feed as-fed) at the expense of FM and subsequently PP (Table 1), corresponding to 11.2, 22.1, and 35.9%, respectively, of MCP CP of total CP. The WinOpti feed optimization program (Agrovision, Apeldoorn, Netherlands) was used to formulate the diets to have roughly the same contents per kg DM of net energy, starch, fibre, and digestible CP, Lys, Met, Thr, Trp and Val, based on previously determined coefficients of standardised ileal digestibility (CSID) for MCP (Rønn et al., 2022), whereas CSID values for FM and PP were derived from the Danish feedstuff table for pigs (Fodervaerktoejer, 2021). Micromineral and vitamins were provided in a premix (Danish Agro a.m.b.a, Karise, Denmark). All diets were pelleted (diameter 3.8 mm) and produced at a commercial feed factory (Danish Agro, Karise, Denmark). The nutrient composition of the diets was analysed at Agrolab Lufa GmbH (Kiel, Germany) (Supplementary Table S1). The piglets were gradually

Table 1
Ingredient composition of the experimental diets for piglets during the first 15 days after weaning containing increasing amounts of microbial cell protein (MCP) at the expense of fishmeal (FM) and/or refined potato protein (PP).

	Dietary inclusion of MCP			
Item	0%	3%	6%	10%
Fixed ingredients (% as-fed)				
Wheat, heat treated	50.00	50.00	50.00	50.00
Whey powder	8.00	8.00	8.00	8.00
Dextrose	4.92	4.92	4.92	4.92
Unrefined potato protein	1.50	1.50	1.50	1.50
Formic and benzoic acids (2:1)	1.50	1.50	1.50	1.50
Yeast products	1.10	1.10	1.10	1.10
Sugar beet molasses	1.00	1.00	1.00	1.00
Vitamin-mineral premix	0.40	0.40	0.40	0.40
Flavours	0.27	0.27	0.27	0.27
Guanidinoacetate	0.10	0.10	0.10	0.10
Phytase	0.04	0.04	0.04	0.04
Variable ingredients (% as-fed)				
MCP[1]	0.00	3.00	6.00	10.00
PP[1]	6.32	7.44	7.53	5.20
FM[1]	5.00	2.00	0.00	0.00
Barley	8.43	5.00	2.00	2.00
Oats	3.00	3.00	2.00	3.00
Wheat	1.48	3.18	5.71	2.69
Fatty acid distillate (palm fat)	2.50	2.78	2.88	3.29
Monocalciumphosphate	1.50	1.63	1.70	1.53
Calcium carbonate	0.00	0.13	0.25	0.29
Lysine sulphate	1.18	1.21	1.26	1.32
Methionine	0.26	0.27	0.28	0.30
Threonine	0.46	0.45	0.45	0.48
Tryptophan	0.19	0.17	0.15	0.13
Valine	0.27	0.25	0.24	0.27
Sodium chloride	0.61	0.68	0.72	0.69

[1] Protein ingredients in the diets MCP, PP, and FM were microbial cell protein product, Uniprotein® (Unibio Group, Roskilde, Denmark); refined potato protein product, Protastar (Scagro A/S, Gesten Denmark); fish meal product, FF Classic (FF Skagen, Skagen, Denmark).

exposed to the experimental diets over the first three days postweaning.

From day 16 postweaning, all piglets were on the same diets, namely Grow10-15 (Net energy, MJ/kg feed as-is (FU/kg)): 8.12 (1.10), CP: 18.28%, digestible CP: 158.53 g/kg, digestible lysine: 12.63 g/kg and Grow15-30 (Net energy, MJ/kg feed as-is (FU/kg)): 7.97 (1.08), CP: 18.21%, digestible CP: 158.22 g/kg, digestible lysine: 12.55 g/kg (Danish Agro, Karise, Denmark) from days 16 to 30 and days 31 to 43 postweaning, respectively (Supplementary Table S2).

The piglets were not supplemented with medicinal zinc at any time.

The piglets were weighed at weaning and at the end of each feeding period. All pigs in one pen were weighed as a unit. Whenever a pig was taken out of the study due to death or disease, the weight was recorded.

Pens were examined daily by the farm staff for any signs of diseased or dead piglets, and the cause was noted, when it could be determined. In case of diarrhoea, animals were treated individually unless more than six pigs in a pen were affected then mass medication was allowed. Due to a miscommunication, a deviation from this routine was made during the first insertion of piglets, where all pens received a diarrhoea preventive antibiotic treatment administered in the water (doxycycline, Doxx-Sol 500 mg/g, Huvepharma, Antwerp, Belgium) following the herd management routines. In the following batches, the piglets were treated individually by injection (oxytetracycline chloride, Alamycin Vet, 100 mg/ml, ScanVet Animal Health, Fredensborg, Denmark).

Intestinal sampling from subgroup of pigs

In three of the seven batches of inserted piglets, one pig was randomly chosen from each pen to be autopsied on day 15 after weaning. In batch 3 and batch 5, one pig was selected from each side of the double pens (16 pigs per batch, four piglets per treatment) whereas in batch 7, pigs were selected from the right side of the double pens only (eight pigs, two piglets per treatment). In total, 10 pigs per treatment were autopsied.

Piglets were sacrificed by captive bold stunning followed by exsanguination. The abdominal cavity was opened, and the entire gastrointestinal tract was removed. The length and weight of the small intestine were measured; the stomach, the large intestine (caecum and colon) and the liver were weighed. The gastrointestinal tract was weighed full and empty. Pieces (3–4 cm long) of intestinal tissue were sampled for intestinal morphology at mid-jejunum, 10 cm proximal to the ileocecal junction, and at the apex of the colon spiral. The intestinal tissue ring was carefully cut open along the line of mesentery attachment, gently rinsed for intestinal contents by flushing with saline, then pinned in each corner to a wax plate and submerged to be fixed in 10% buffered formalin.

Intestinal morphology and histopathology

The tissue samples were dehydrated and embedded in paraffin wax. A slide was prepared from each sample containing a minimum of four sections cut at 4 μm, at least 50 μm apart. All slides were stained with haematoxylin and eosin (HE). Histological analysis of mid-jejunum, ileum, and colonic tissue was conducted at the commercial laboratory ALAB bioscience (Warsaw, Poland) using a standard light microscope, ZEISS Axiolab 5, and Zen Blue 3.0 software (Carl Zeiss Microscopy, Oberkochen, Germany). In jejunal and ileal sections, the height of villi and depth of crypts were assessed, 50–60 villi and 50–60 corresponding crypts were measured in 5–6 different histological sections at ×10 objective magnification for each sample. Furthermore, height of the enterocytes, number of infiltrating epithelium lymphocytes and number

of goblet cells per 100 enterocytes were recorded. The same parameters were recorded for colonic tissue except for the height of villi. We assessed the severity of histopathological changes for each identified pathological change in the preparation on a scale of 0–4 according to the formula routinely used in non-clinical pathology 0 – no change, 1 – minimal, 2 – mild, 3 – moderate, 4 – significant or four-point scale: 0 – Normal, 1 – Low, 2 – Moderate, and 3 – Severe (Schafer et al., 2018). The following parameters were graded: extent of infiltration of the stromal mucosa with lymphocytes, intestinal blunting, cell detritus enrichment with eosinophils, signs of erosion/ulcer, severity of oedema in stromal mucosa, degree of vessel dilatation in stromal mucosa of villi, infiltration of the submucosa with lymphocytes, severity of oedema of the submucosa, occurrence of single-cell necrosis/apoptosis, and degree of hyperplasia of enterocytes. Evaluation of the gut-associated lymphoid tissue (GALT) included the numbers of lymphoid follicles visible in the intestinal section. All microscopic evaluations were performed in a blinded fashion.

Calculations and statistical analyses

Average daily gain (**ADG**) per piglet was calculated as the difference in weight of piglets at insertion in the pen and total piglet weight at exit of each feeding phase divided by the number of pigs in each pen and the number of days in each phase. The ADG in the overall test period from weaning to end of the trial was likewise calculated as the difference in weight at insertion and at exit of the trial divided by the number of pigs and days in test. When a pig was taken out of the trial due to disease or death, the number of pigs and days in each phase was adjusted (only the number of days that the piglet was in test was used). The weight of piglets taken out of test was included in the pen weight at exit. Average daily feed intake (**ADFI**) was calculated as the amount of feed provided per feed dispenser in each phase (or the total test period) minus the remaining feed residues in each of the feeding phases. When a pig was taken out of the trial, the days in each phase was adjusted (only the number of days that the piglet was in test was used). Feed Conversion Ratio (**FCR**) was calculated as FI (g/day) divided by ADG (g/day).

Data for ADG, ADFI, FCR were analysed using the mixed procedure in R, where diet was a fixed effect, and average weight of piglets, when included, and number of piglets in the pen were covariates, and batch and repetition within a batch were considered random effects. The weight and relative weight of the liver, length of the small intestine and weight of full and empty GI-tract were analysed using the mixed procedure in SAS where diet and sex were fixed effects and batch was considered a random effect. PROC GLM in SAS and orthogonal polynomial contrast coefficients were used to determine linear and quadratic effects of MPC level in the diets on the VO weight and small intestinal length. Data for intestinal morphology (villus height, crypt depth, villus height to crypt depth ratio), height of epithelial cells, infiltrating epithelial lymphocytes, and number of goblet cells in the small intestine were analysed as repeated measurements using the mixed procedure in SAS where diet and segment and their interaction were between-animal effects, segment was within-animal effect, and block was a random effect. The same model was used in colon without the repeated statement.

Histopathological data were analysed in R (4.2.1) using a Kruskal-Wallis rank sum test (Kassambara, 2020) with diet as an independent variable. A Dunn's test with Bonferroni adjustment was used to make pairwise comparisons between the mean ranks of diets.

Results are expressed as least square means and variance as SEM. Significance was considered at $P < 0.05$ and tendencies between $0.05 < P < 0.10$.

Results

Growth performance and indicators of piglet health

Increasing the inclusion of MCP in the diet during day 0–15 postweaning tended ($P = 0.09$) to reduce daily gain in piglets fed 10% MCP (Table 2). Feed intake during this period was not affected but FCR turned out to be significantly higher ($P < 0.01$) in piglets fed the highest inclusion rate of MCP (10% MCP) than in piglets fed 0 or 3% MCP. The higher FCR of the piglets fed 10% MCP did not persist into the following phases where no differences in daily

Table 2
Impact of increasing dietary inclusion of microbial cell protein (MCP) on average daily gain, average daily feed intake, and feed conversion ratio (feed intake/daily gain) in piglets during the first 15 days after weaning, and during days 15–30 and days 30–43 after changing to grower diets.

	Dietary inclusion of MCP					*P*-value		
Item	0%	3%	6%	10%	SEM	Diet	Linear	Quadratic
Start weight (kg)	6.38	6.37	6.35	6.37				
Final weight (kg)	17.09	17.77	17.80	17.71				
Days 0–15								
Daily gain (kg/d)	0.154	0.159	0.152	0.145	0.005	0.088	0.580	0.126
Feed intake (kg/d)[1]	0.216	0.219	0.219	0.214	0.004	0.733	0.962	0.293
Feed conversion ratio	1.406[a]	1.392[a]	1.447[ab]	1.487[b]	0.016	0.003	0.317	0.161
Days 15–30[2]								
Daily gain (kg/d)	0.394	0.391	0.400	0.403	0.015	0.656	0.667	0.652
Feed intake (kg/d)[1]	0.562	0.562	0.568	0.560	0.009	0.954	0.519	0.625
Feed conversion ratio	1.435	1.444	1.433	1.400	0.037	0.392	0.975	0.275
Days 30–43[3]								
Daily gain (kg/d)	0.656	0.709	0.713	0.708	0.023	0.136	0.608	0.097
Feed intake (kg/d)[1]	1.019	1.054	1.075	1.060	0.016	0.119	0.724	0.084
Feed conversion ratio	1.576	1.504	1.524	1.501	0.047	0.556	0.431	0.517
Overall								
Daily gain (kg/d)	0.396	0.413	0.415	0.413	0.009	0.255	0.690	0.142
Feed intake (kg/d)[1]	0.591	0.603	0.611	0.602	0.007	0.417	0.692	0.166
Feed conversion ratio	1.497	1.462	1.477	1.461	0.024	0.635	0.385	0.652

Values are least-square means and SEM of 14 observations for all treatments.
[a,b] Values within a row with different superscripts differ, $P < 0.05$.
[1] Feed intake is expressed on an as-fed basis.
[2] Piglets were fed Grow10-15 (Danish Agro, Roskilde, Denmark).
[3] Piglets were fed Grow15-30 (Danish Agro, Roskilde, Denmark).

gain, feed intake, and feed conversion ratio in either grower phase 1 (15–30 days) or phase 2 (30–43 days) were found. The performance during the entire experimental period (days 0–43) was not affected by the inclusion of MCP during days 0–15.

Inclusion of MCP in the diet did not have any influence on the recorded incidences of diarrhoea during the first two weeks post-weaning (Supplementary Table S3). During the following phases, days 15–30 and days 30–43, no incidences of diarrhoea were recorded. The mortality was low (1%) during the experiment with deaths equally distributed among treatments and periods (Supplementary Table S3).

Organ measurements and intestinal morphology and histopathology

The organ weights and relative liver weight presented as percentage of BW are summarised in Table 3. Neither the liver weight nor the length or weight of the individual segments of the gastrointestinal tract were influenced by increasing inclusion of dietary MCP ($P > 0.05$).

In the small intestine, villous height tended ($P = 0.09$) to show a quadratic response to level of MCP in the diet with the longest villi found in piglets fed 6% MCP. In contrast, no effect of increasing dietary inclusion of MCP was seen on crypt depth (Table 4). The VC ratio showed a quadratic response to increased dietary inclusion of MCP ($P = 0.02$) and a tendency ($P = 0.06$) for the effect of diet on VC ratio was observed with piglets fed 6% MCP having the highest VC ratio. The dietary treatments did not affect enterocyte height, number of goblet cells or intraepithelial lymphocytes ($P > 0.05$). For villous height, crypt depth, and number of goblet cells, there was a significant effect of segment ($P < 0.05$). In the colon, increasing dietary inclusion of MCP had no effect on the morphological parameters measured.

The histopathological parameters, where increasing dietary inclusion of MCP tended ($P < 0.10$) to have an effect, are shown in Fig. 1. Data are visualised as bar plots of raw counts of observed scores. Figures include χ^2 test-statistic and their associated *P*-values of the effect of dietary MCP inclusion on sum of ranks. For all other histopathological parameters assessed, no effect of treatment was observed. In jejunum, piglets fed 10% MCP tended to have a different population median for oedema of the stromal mucosa (a) compared with piglets fed 0% MCP. Raw data show more frequent observations of low scores for piglets fed 10% MCP. Likewise, piglets fed 10% MCP had a higher incidence of low score (0) for infiltration of the stromal mucosa in colon (b) than piglets fed 0% MCP, and hence, the population median tended to differ between 0 and 10% MCP. Furthermore, the diet tended to affect the population distribution of infiltration of the submucosa in colon (c). Piglets fed 3% MCP deviated from 10% MCP. Piglets fed 3% MCP were uniformly distributed among the scores whereas piglets in the 10% MCP group were solely given score of 0 or 1.

Table 3
Impact of increasing dietary inclusion of microbial cell protein (MCP) on liver weight, small intestinal length, and weight of full and empty gastrointestinal tract of piglets (n = 10).

	Dietary inclusion of MCP					*P*-value[1]
Item	0%	3%	6%	10%	SEM	
Liver weight (g)	280	284	283	224	35	0.32
Relative liver weight (g/kg)	31.0	32.7	33.1	29.1	1.8	0.32
Small intestine, length (m)	10.4	9.9	10.0	9.5	0.5	0.66
Weight of GI-tract, full (g)						
Stomach	215	263	219	199	31	0.49
Small intestine	644	634	622	661	46	0.94
Large intestine[2]	334	316	378	330	31	0.39
Weight of GI-tract, empty (g)						
Stomach	64.6	66.3	70.3	65.3	4.1	0.74
Small intestine	442	436	425	447	34	0.96
Large intestine[2]	150	153	169	140	14	0.43

Abbreviation: GI = gastrointestinal.
[1] No linear or quadratic effects of dietary inclusion of MCP were observed.
[2] Caecum and colon.

Table 4
Impact of increasing dietary inclusion of microbial cell protein (MCP) on morphology in jejunum, ileum, and colon of piglets.

	Dietary inclusion of MCP					Segment			*P*-values			
Item	0%	3%	6%	10%	SEM	Jejunum	Ileum	SEM	Diet[1]	Segment	Lin[2]	Quad
Small intestine												
Villi height, µm	334	342	361	315	13	371	304	10	0.12	<0.0001	0.55	0.09
Crypt depth, µm	251	249	247	268	10	267	241	8	0.30	0.01	0.17	0.19
VC ratio[3]	1.35[ab]	1.39[ab]	1.51[a]	1.18[b]	0.08	1.43	1.28	0.06	0.06	0.06	0.21	0.02
Enterocytes height, µm	28.8	29.1	28.4	29.7	1.70	28.0	30.0	1.5	0.93	0.09	0.89	0.54
Goblet cells[4]	1.69	1.94	2.41	1.75	0.35	0.55	3.35	0.27	0.33	<0.0001	0.97	0.22
Infiltrating epithelium lymphocytes[5]	13.7	14.3	15.1	15.8	1.20	15.10	14.30	3.30	0.62	0.21	0.12	0.83
Colon												
Crypt depth, µm	472	480	488	412	29				0.22		0.14	0.14
Enterocytes height, µm	21.7	21.3	21.9	21.9	1.5				0.90		0.62	0.73
Goblet cells[4]	21	22	18	23	2.0				0.12		0.63	0.19
Infiltrating epithelium lymphocytes[5]	1.97	1.97	1.97	2.05	0.31				0.87		0.50	0.66

[a,b] Values within a row with different superscripts differ, $P < 0.05$.
[1] Diet × segment interaction was not observed for any of the variables ($P > 0.05$).
[2] Orthogonal polynomial contrast coefficients were used to determine linear (Lin) and quadratic (Quad) effects of dietary inclusion of MCP.
[3] VC ratio = Villous height to crypt depth ratio.
[4] Goblet cells per 100 enterocytes.
[5] Intraepithelial lymphocytes per 100 enterocytes.

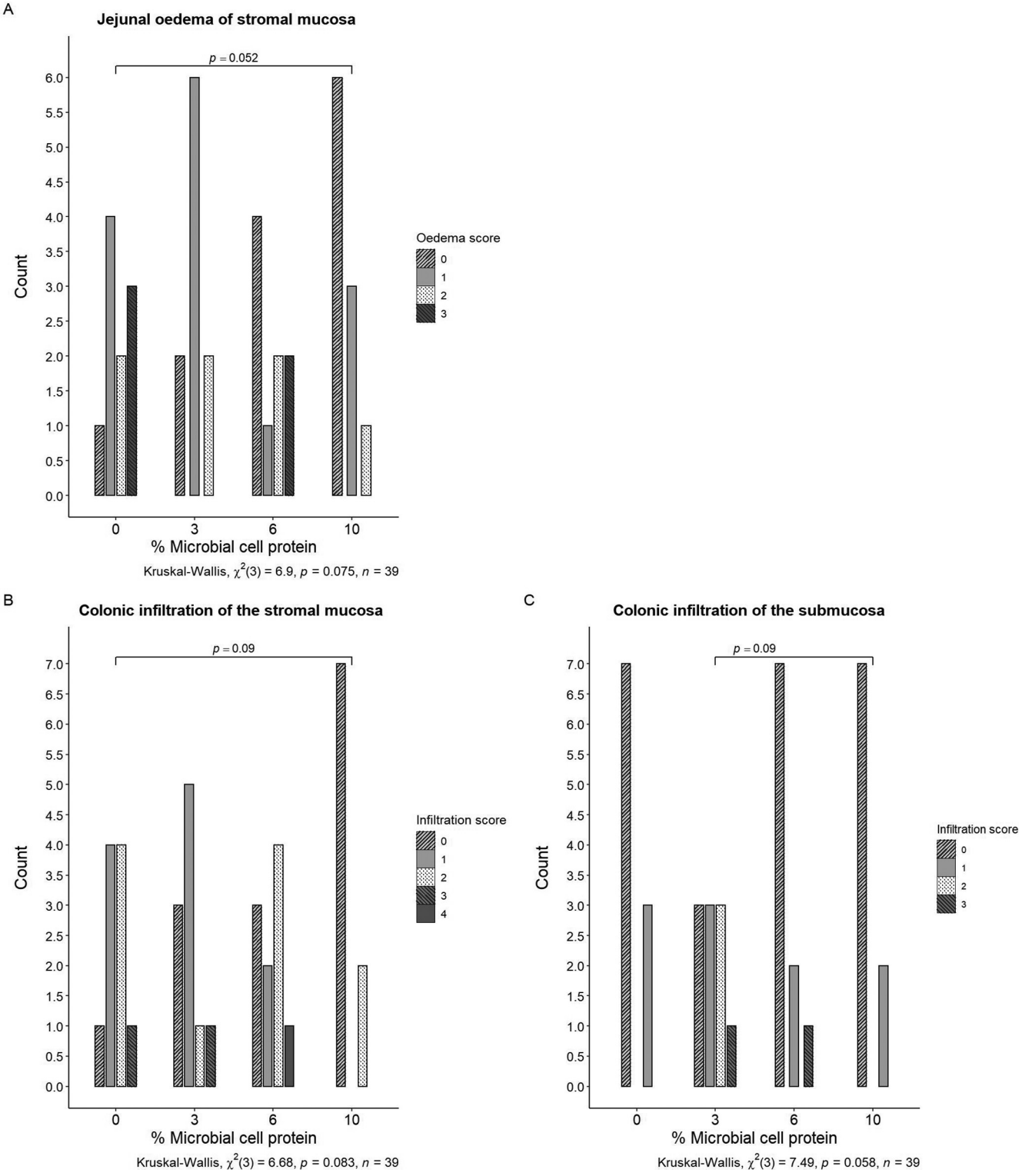

Fig. 1. Impact of increasing dietary inclusion of microbial cell protein (MCP) on (a) oedema of the stromal mucosa in jejunum, (b) infiltration of the stromal mucosa in colon, and (c) infiltration of the submucosa in colon of piglets. Histological parameters were scored on a score from 0 to 4 where 0 designate normal/healthy, 1 low level of changes, 2 mild changes, 3 moderate changes, and 4 severe changes. For a detailed description of the scores, see Supplementary Material S1.

Discussion

There is a need for new quality protein sources for pig production to improve the sustainability of production. Particularly for weaning piglets, it is important that the new protein sources are palatable, digestible, exerting no negative effects during the vulnerable postweaning feeding period, and they must be competitive in terms of price with other quality protein feeds (Pluske et al., 2002; Wu et al., 2015; Li et al., 2021). The present study showed sustained growth performance, when including up to 6% MCP in the postweaning diet. In contrast to this, Zhang et al. (2013) showed that 5% dietary inclusion of MCP at the expense of fish

meal reduced FCR, whereas the inclusion of 2.5% MCP had no adverse effect on FCR in a four-week feeding trial with piglets weaned at four weeks of age. In a trial with weaned pigs (35 days, 10.4 kg) fed diets where MCP substituted soybean meal, fish meal, and meat and bone meal in the control diet, for four weeks, the inclusion of up to 12% MCP in the diet had positive effects on ADFI and ADG (Øverland et al., 2001); however, this was without any effect on FCR. Other types of MCP, e.g. *Corynebacterium glutamicum* cell mass (Cheng et al., 2021) and lysine cell mass (Hong et al., 2021), have been studied and can be included up to 2.1 and 2.8%, respectively, in the early postweaning period without affecting the growth performance. These trials were all small-scale trials with a limited number of animals, whereas the present study demonstrates production-scale usability of MCP and, furthermore, indicates that MCP may constitute up to 6% of diets at the expense of FM and PP during the first two weeks postweaning in commercial pig production. The performance data in the following periods (15–30 days and 30–43 days) were not affected by the dietary treatments in the early postweaning phase indicating that the inclusion of MCP did not have any long-term effects on gastrointestinal health or digestive performance.

The weight of the visceral organs (**VO**) may be dependent on the diet composition (Nyachoti et al., 2000) and especially for the liver, the content of potentially toxic components in a diet may increase liver weight (Greaves, 2012). Assessment of the impact of nutritional treatment on the VO weight is widely used to ensure that the physiological needs of the animal are fulfilled and that the treatment has no toxicological effects (Elefson et al., 2021). In the present study, the liver weight was not influenced by the increasing inclusion of MCP in the diet, which suggests that MCP does not contain components eliciting a toxicological reaction in piglets, to ascertain this does, however, more thorough investigations are required. The relative liver weight was within the range previously observed for pigs at the same age (Elefson et al., 2021), and hence, the MCP-supplemented diets supported normal growth of the liver. The length and weight of the gastrointestinal tract are indicative of the digestive and absorptive capacity (Wang et al., 2020). Provision of MCP in the diet to weaned pigs in the present experiment did not induce any changes in the dimensions of the gastrointestinal tract and the weight and length were within the ranges previously observed for pigs two weeks postweaning (Elefson et al., 2021).

In the present study, neither of the diets fed to piglets pre- or postweaning were supplemented with medicinal zinc. Yet, the incidence of diarrhoea was very low in all dietary treatment groups showing that MCP can replace FM and subsequently PP, with up to 36% of CP in diets in the immediate postweaning period without increasing the risk of diarrhoea. This is an important observation, since the use of dietary zinc oxide addition to prevent postweaning diarrhoea (Heo et al., 2010) has been banned in the EU from June 2022 (EU, 2016). However, the present experiment was performed on a high-sanitary farm which reduces the overall risk of diarrhoea (Jayaraman and Nyachoti, 2017).

We have previously reported that MCP has lower coefficients of standardised ileal digestibility for CP and individual essential amino acids (**EAAs**) than FM and PP (Rønn et al., 2022). When comparing the digestibility of MCP and FM, Zhang et al. (2013) also found higher digestibilities for EAAs in FM. However, low digestibilities in MCP are to a great extent compensated by high concentrations of EAAs, whereby MCP fulfilled the Danish recommendations for protein, expressed as the g digestible EAA per kg as-fed relative to that of Lys (Rønn et al., 2022). MCP was particularly rich in digestible Trp and Met relative to Lys, and superior to FM with respect to recommendations for all EAAs, except Thr and Phe.

Due to the lower digestibility of MCP, more protein was added to the MCP-containing diets, which is reflected in the content of CP. The difference in CP, 18.3 vs 20%, was too small to have any impact on the health of the pigs in accordance with (Htoo et al., 2007). The growth of the animals was not affected by the inclusion of MCP in the diets supporting the observation that MCP can fulfil requirements for EAA and constitute 22% of dietary protein in diets for newly weaned piglets without negative impact on growth when the minimum requirements for EAA are fulfilled. A recent study showed that protein digestibility is very low in the early postweaning period irrespective of the protein source (Engelsmann et al., 2022) suggesting that protein digestibility might not differ between dietary treatments in the first period (days 1–15) in the present experiment. Flow of undigested protein to the hindgut has been suggested to be a contributing factor to postweaning diarrhoea (Heo et al., 2013). The very low levels of diarrhoea indicated that this was not an issue in the present study. However, this study was performed on a high-sanitary farm, and it cannot be excluded that the results would have been different in a low-sanitary environment.

Diets did not affect villus height and crypt depth two weeks after weaning. Negative effects of weaning are generally observed for intestinal morphology (Hedemann et al., 2003); however, two weeks after weaning, villus height has usually recovered (Burrin and Stoll, 2003; Engelsmann et al., 2022). The VC ratio provides an indication of the absorptive area of the small intestine (Montagne et al., 2003) and in the present study, the highest VC ratio parallels the lowest FCR which is seen in the groups fed 0, 3, or 6% MCP. A similar correlation was previously observed (Zhang et al., 2013), when 2.5% of MCP was included in diets for weaned pigs. In contrast to this, the inclusion of *Corynebacterium glutamicum* cell mass tended to linearly reduce VC ratio (Cheng et al., 2021). The relatively high content of nucleic acids in MCP has been considered its main anti-nutritional factor (Bratosin et al., 2021); however, optimisation of the processing of MCP has reduced the content of nucleic acids by nearly 90% (Strong et al., 2015). In other single-cell protein products, e.g. yeast, the content of nucleic acids is considered a benefit that may exert positive effects on the intestinal mucosa (Håkenåsen et al., 2020), and the same may apply to MCP. Positive effects of adding nucleotides to diet for weaning pigs have been observed in several studies (Zheng et al., 2021) and the nucleotides in MCP may have affected intestinal health positively in the present study.

Increasing the inclusion of MCP tended to improve few indicators of gastrointestinal health in jejunum and colon. The stromal mucosa in jejunum and colon was graded as more healthy in pigs fed 10% MCP with less oedema and infiltration of the stromal mucosa. Stromal cells are located beneath the epithelial cells and are important for maintaining the integrity of the intestinal mucosal barrier and have multiple roles in immune responses and inflammation (Owens and Simmons, 2013; Kurashima et al., 2019) and nucleotides have been suggested to enhance the development of the immune system (Liu et al., 2018). Piglets fed 10% MCP had the numerically highest prevalence of mucosal epithelium with no pathological changes in the colon (results not shown). This indicates that the inclusion of 10% MCP in the diet in the early postweaning period improved the provision of building blocks necessary for maintaining intestinal integrity to the piglets. Nucleotides are needed for enterocyte proliferation and capacity for *de novo* synthesis may be insufficient in the early postweaning period (Sauer et al., 2011). The result of the present study indicates that despite slightly poorer growth performance, the diet providing 10% MCP had the most optimal composition for developing and maintaining intestinal integrity. Interactions between the microbiota and the intestinal mucosa are important for intestinal integrity (Schiffrin and Blum, 2002), and a recent study showed that the colonic microbiota and its metabolites were involved in adaptive alterations of the colon crypts without effect on the histopathological assessment (Iakhno et al., 2020).

In conclusion, this study demonstrated that MCP could constitute 6% of diets as-fed (22% of total CP) for newly weaned piglets without negative effects on growth rates and FCR, and dietary inclusion of MCP even tended to improve VC ratio. Higher inclusion rate of MCP (10% MCP in feed as-fed) tended to improve indicators of gastrointestinal (particularly hind-gut) health with no overall negative impact on growth performance over the entire weaner period, but the FCR was significantly increased over the first 15 days where this MCP-supplemented diet was fed. Overall, the study shows that the inclusion of MCP in diets for newly weaned piglets could be part of improving the sustainability of pig production without negative effect on growth or intestinal health – also when the use of medicinal zinc is abandoned.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.100798.

Ethics approval

The study complied with the guidelines of the Danish Ministry of Justice, Act no. 474 of May 15, 2014 concerning experiments with animals and care of experimental animals.

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available from the authors upon request.

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Declaration of interest

The present study was part of a larger project financially supported by the national Green Development and Demonstration Program (GUDP grant no.: 34009-19-1544 of 17 June 2019), Denmark. Two private companies supplied the protein sources used in this study: The MCP product was supplied by Unibio A/S, Roskilde, Denmark, and the FM, PP and SPC products were supplied Danish Agro a.m.b.a., Køge, Denmark. Danish Agro formulated and produced the four diets used in the experiment. Special thanks are also due to TestGris project leaders Niels Ove Nielsen and Dorthe Carlsson, the Danish Pig Advisory Center, Herning, Denmark, and all staff at the pig facility Skjoldborg, Herning, Denmark, for expert care of the animal and assistance in during sampling of the pigs.

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References

Anupama, Ravindra, P., 2000. Value-added food: Single cell protein. Biotechnology Advances 18, 459–479. https://doi.org/10.1016/S0734-9750(00)00045-8.
Bratosin, B.C., Darjan, S., Vodnar, D.C., 2021. Single Cell Protein: A Potential Substitute in Human and Animal Nutrition. Sustainability 13, 9284.
Burrin, D., Stoll, B., 2003. Weaning the pig: concepts and consequences. In: Pluske, J. R., Le Dividich, J., Verstegen, M.W.A. (Eds.), Intestinal nutrient requirements in weanling pigs. Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 301–335.
Cheng, Y.C., Duarte, M.E., Kim, S.W., 2021. Nutritional and functional values of lysed Corynebacterium glutamicum cell mass for intestinal health and growth of nursery pigs. Journal of Animal Science 99, 1–15. https://doi.org/10.1093/jas/skab331.
Elefson, S.K., Lu, N., Chevalier, T., Dierking, S., Wang, D., Monegue, H.J., Matthews, J. C., Jang, Y.D., Chen, J., Rentfrow, G.K., Adedokun, S.A., Lindemann, M.D., 2021. Assessment of visceral organ growth in pigs from birth through 150 kg. Journal of Animal Science 99, 1–11. https://doi.org/10.1093/jas/skab249.
Engelsmann, M.N., Jensen, L.D., van der Heide, M.E., Hedemann, M.S., Nielsen, T.S., Nørgaard, J.V., 2022. Age-dependent development in protein digestibility and intestinal morphology in weaned pigs fed different protein sources. Animal 16, 100439. https://doi.org/10.1016/j.animal.2021.100439.
EU, 2016. Commission implementing regulation (EU) 2016/1095 of 6 July 2016 concerning the authorisation of Zinc acetate dihydrate, Zinc chloride anhydrous, Zinc oxide, Zinc sulphate heptahydrate, Zinc sulphate monohydrate, Zinc chelate of amino acids hydrate, Zinc chelate of protein hydrolysates, Zinc chelate of glycine hydrate (solid) and Zinc chelate of glycine hydrate (liquid) as feed additives for all animal species and amending Regulations (EC) No 1334/2003, (EC) No 479/2006, (EU) No 335/2010 and Implementing Regulations (EU) No 991/2012 and (EU) No 636/2013. Official Journal of the European Union L 182, 7.
Fodervaerktoejer, 2021. Landbrug og Fødevarer, Sektor for Gris [On-line] (In Danish). Available from: https://svineproduktion.dk/Viden/Paa-kontoret/Oekonomi_ledelse/Beregningsvaerktoejer/Fodervaerktoejer. Retrieved on 12 March 2021.
Greaves, P., 2012. Chapter 9 – Liver and Pancreas. In Histopathology of Preclinical Toxicity Studies (Fourth Edition) (ed. Greaves, P.), Academic Press, Boston, MA, USA. pp. 433–535. doi: https://doi.org/10.1016/B978-0-444-53856-7.00009-9.
Håkenåsen, I.M., Øverland, M., Ånestad, R., Åkesson, C.P., Sundaram, A.Y.M., Press, C. M., Mydland, L.T., 2020. Gene expression and gastrointestinal function is altered in piglet small intestine by weaning and inclusion of Cyberlindnera jadinii yeast as a protein source. Journal of Functional Foods 73, 104118. https://doi.org/10.1016/j.jff.2020.104118.

Hedemann, M.S., Højsgaard, S., Jensen, B.B., 2003. Small intestinal morphology and activity of intestinal peptidases in piglets around weaning. Journal of Animal Physiology and Animal Nutrition 87, 32–41.

Hellwing, A.L., Tauson, A.H., Kjos, N.P., Skrede, A., 2007. Bacterial protein meal in diets for growing pigs: effects on protein and energy metabolism. Animal 1, 45–54. https://doi.org/10.1017/S1751731107283879.

Heo, J.M., Kim, J.C., Hansen, C.F., Mullan, B.P., Hampson, D.J., Maribo, H., Kjeldsen, N., Pluske, J.R., 2010. Effects of dietary protein level and zinc oxide supplementation on the incidence of post-weaning diarrhoea in weaner pigs challenged with an enterotoxigenic strain of Escherichia coli. Livestock Science 133, 210–213. https://doi.org/10.1016/j.livsci.2010.06.066.

Heo, J.M., Opapeju, F.O., Pluske, J.R., Kim, J.C., Hampson, D.J., Nyachoti, C.M., 2013. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. Journal of Animal Physiology and Animal Nutrition 97, 207–237. https://doi.org/10.1111/j.1439-0396.2012.01284.x.

Hong, J., Kim, H.-S., Do, S., Kim, H.-J., Kim, S.-W., Jang, S.-K., Kim, Y.-Y., 2021. Effects of Lysine Cell Mass Supplementation as a Substitute for L-Lysine·HCl on Growth Performance, Diarrhea Incidence, and Blood Profiles in Weaning Pigs. Animals 11, 2092.

Htoo, J.K., Araiza, B.A., Sauer, W.C., Rademacher, M., Zhang, Y., Cervantes, M., Zijlstra, R.T., 2007. Effect of dietary protein content on ileal amino acid digestibility, growth performance, and formation of microbial metabolites in ileal and cecal digesta of early-weaned pigs. Journal of Animal Science 85, 3303–3312. https://doi.org/10.2527/jas.2007-0105.

Iakhno, S., Umu, Ö.C.O., Håkenåsen, I.M., Åkesson, C.P., Mydland, L.T., Press, C.M., Sørum, H., Øverland, M., 2020. Effect of Cyberlindnera jadinii yeast as a protein source on intestinal microbiota and butyrate levels in post-weaning piglets. Animal Microbiome 2, 13. https://doi.org/10.1186/s42523-020-00031-x.

Jayaraman, B., Nyachoti, C.M., 2017. Husbandry practices and gut health outcomes in weaned piglets: A review. Animal Nutrition 3, 205–211. https://doi.org/10.1016/j.aninu.2017.06.002.

Kassambara, A., 2020. Pipe-Friendly Framework for Basic Statistical Tests. Retrieved on 15 August 2022 from https://CRAN.R-project.org/package=rstatix.

Kuhad, R.C., Singh, A., Tripathi, K.K., Saxena, R.K., Eriksson, K.E., 1997. Microorganisms as an alternative source of protein. Nutrition Reviews 55, 65–75. https://doi.org/10.1111/j.1753-4887.1997.tb01599.x.

Kurashima, Y., Tokuhara, D., Kamioka, M., Inagaki, Y., Kiyono, H., 2019. Intrinsic Control of Surface Immune and Epithelial Homeostasis by Tissue-Resident Gut Stromal Cells. Frontiers in Immunology 10, 1281. https://doi.org/10.3389/fimmu.2019.01281.

Li, Y., Liu, Y., Wu, J., Chen, Q., Zhou, Q., Wu, F., Zhang, R., Fang, Z., Lin, Y., Xu, S., Feng, B., Zhuo, Y., Wu, D., Che, L., 2021. Comparative effects of enzymatic soybean, fish meal and milk powder in diets on growth performance, immunological parameters, SCFAs production and gut microbiome of weaned piglets. Journal of Animal Science and Biotechnology 12, 106. https://doi.org/10.1186/s40104-021-00625-8.

Liu, Y., Espinosa, C.D., Abelilla, J.J., Casas, G.A., Lagos, L.V., Lee, S.A., Kwon, W.B., Mathai, J.K., Navarro, D.M.D.L., Jaworski, N.W., Stein, H.H., 2018. Non-antibiotic feed additives in diets for pigs: A review. Animal Nutrition 4, 113–125. https://doi.org/10.1016/j.aninu.2018.01.007.

Maiuolo, J., Oppedisano, F., Gratteri, S., Muscoli, C., Mollace, V., 2016. Regulation of uric acid metabolism and excretion. International Journal of Cardiology 213, 8–14. https://doi.org/10.1016/j.ijcard.2015.08.109.

Matassa, S., Boon, N., Pikaar, I., Verstraete, W., 2016. Microbial protein: future sustainable food supply route with low environmental footprint. Microbial Biotechnology 9, 568–575. https://doi.org/10.1111/1751-7915.12369.

Montagne, L., Pluske, J.R., Hampson, D.J., 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Animal Feed Science and Technology 108, 95–117.

Nyachoti, C.M., de Lange, C.F.M., McBride, B.W., Leeson, S., Schulze, H., 2000. Dietary influence on organ size and in vitro oxygen consumption by visceral organs of growing pigs. Livestock Production Science 65, 229–237. https://doi.org/10.1016/S0301-6226(00)00157-3.

Øverland, M., Skrede, A., Matre, T., 2001. Bacterial Protein Grown on Natural Gas as Feed for Pigs. Acta Agriculturae Scandinavica, Section A – Animal Science 51, 97–106. https://doi.org/10.1080/090647001750193422.

Øverland, M., Petter, N.K., Skrede, A., 2004. Effect of bacterial protein meal grown on natural gas on growth performance and carcass traits of pigs. Italian Journal of Animal Science 3, 323–336. https://doi.org/10.4081/ijas.2004.323.

Øverland, M., Tauson, A.-H., Shearer, K., Skrede, A., 2010. Evaluation of methane-utilising bacteria products as feed ingredients for monogastric animals. Archives of Animal Nutrition 64, 171–189. https://doi.org/10.1080/17450391003691534.

Owens, B.M.J., Simmons, A., 2013. Intestinal stromal cells in mucosal immunity and homeostasis. Mucosal Immunology 6, 224–234. https://doi.org/10.1038/mi.2012.125.

Pluske, J.R., Pethick, D.W., Hopwood, D.E., Hampson, D.J., 2002. Nutritional influences on some major enteric bacterial diseases of pigs. Nutrition Research Reviews 15, 333–371.

Ritala, A., Häkkinen, S.T., Toivari, M., Wiebe, M.G., 2017. Single Cell Protein—State-of-the-Art, Industrial Landscape and Patents 2001–2016. Frontiers in Microbiology 8, 2009. https://doi.org/10.3389/fmicb.2017.02009.

Rønn, M., Thorsteinsson, M., Johannsen, J.C., Nørgaard, J.V., Julegaard, I.K., Nielsen, M.O., 2022. Evaluation of nutritional quality for weaner piglets of a new methanotrophic microbial cell-derived protein feed. Animal Feed Science and Technology 294, 115498. https://doi.org/10.1016/j.anifeedsci.2022.115498.

Sauer, N., Mosenthin, R., Bauer, E., 2011. The role of dietary nucleotides in single-stomached animals. Nutrition Research Reviews 24, 46–59. https://doi.org/10.1017/S0954422410000326.

Schafer, K.A., Eighmy, J., Fikes, J.D., Halpern, W.G., Hukkanen, R.R., Long, G.G., Meseck, E.K., Patrick, D.J., Thibodeau, M.S., Wood, C.E., Francke, S., 2018. Use of Severity Grades to Characterize Histopathologic Changes. Toxicologic Pathology 46, 256–265. https://doi.org/10.1177/0192623318761348.

Schiffrin, E.J., Blum, S., 2002. Interactions between the microbiota and the intestinal mucosa. European Journal of Clinical Nutrition 56, S60–S64. https://doi.org/10.1038/sj.ejcn.1601489.

Skrede, A., Berge, G.M., Storebakken, T., Herstad, O., Aarstad, K.G., Sundstøl, F., 1998. Digestibility of bacterial protein grown on natural gas in mink, pigs, chicken and Atlantic salmon. Animal Feed Science and Technology 76, 103–116. https://doi.org/10.1016/S0377-8401(98)00208-9.

Strong, P.J., Xie, S., Clarke, W.P., 2015. Methane as a Resource: Can the Methanotrophs Add Value? Environmental Science & Technology 49, 4001–4018. https://doi.org/10.1021/es504242n.

Valini, G.A.C., Duarte, M.S., Calderano, A.A., Teixeira, L.M., Rodrigues, G.A., Fernandes, K.M., Veroneze, R., Serão, N.V.L., Mantovani, H.C., Rocha, G.C., 2021. Dietary nucleotide supplementation as an alternative to in-feed antibiotics in weaned piglets. Animal 15, 100021. https://doi.org/10.1016/j.animal.2020.100021.

Wang, M., Yang, C., Wang, Q.Y., Li, J.Z., Li, Y.L., Ding, X.Q., Yin, J., Yang, H.S., Yin, Y.L., 2020. The growth performance, intestinal digestive and absorptive capabilities in piglets with different lengths of small intestines. Animal 14, 1196–1203. https://doi.org/10.1017/S175173111900288X.

Wiederkehr, M.R., Moe, O.W., 2011. Uric Acid Nephrolithiasis: A Systemic Metabolic Disorder. Clinical Reviews in Bone and Mineral Metabolism 9, 207–217. https://doi.org/10.1007/s12018-011-9106-6.

Wu, Y., Jiang, Z., Zheng, C., Wang, L., Zhu, C., Yang, X., Wen, X., Ma, X., 2015. Effects of protein sources and levels in antibiotic-free diets on diarrhea, intestinal morphology, and expression of tight junctions in weaned piglets. Animal Nutrition 1, 170–176. https://doi.org/10.1016/j.aninu.2015.08.013.

Zhang, H.Y., Piao, X.S., Li, P., Yi, J.Q., Zhang, Q., Li, Q.Y., Liu, J.D., Wang, G.Q., 2013. Effects of Single Cell Protein Replacing Fish Meal in Diet on Growth Performance, Nutrient Digestibility and Intestinal Morphology in Weaned Pigs. Asian-Australasian Journal of Animal Science 26, 1320–1328. https://doi.org/10.5713/ajas.2013.13200.

Zheng, L., Duarte, M.E., Sevarolli Loftus, A., Kim, S.W., 2021. Intestinal Health of Pigs Upon Weaning: Challenges and Nutritional Intervention. Frontiers in Veterinary Science 8, 628258. https://doi.org/10.3389/fvets.2021.628258.