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Effects of a high protein starter diet with fermented soybean cake on growth performance of organic pigs weaned outdoor

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HIGHLIGHTS

- A high protein starter diet with fermented soybean cake was tested under organic European conditions.
- Organic pigs weaned outdoor benefitted from being fed the high protein starter diet.
- The high protein starter diet improved daily gain by 8% and feed efficiency by 12%.
- The high protein diet reduced the estimated nitrogen excretion of outdoor organic weaners.
- Subjective evaluation showed no effect of dietary treatment on post-weaning diarrhea.

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ABSTRACT

Weaners have a great genetic potential for growth, which currently are not fully utilized in the organic pig production under European conditions, characterized by prolonged lactation period, access to roughage and a limited range of feedstuff available as compared to conventional pig production. Nutrition is a key element for improving the growth and utilization of nutrients, however, is also a risk factor for post-weaning diarrhea. The objective of this study was to investigate the effect of a starter diet, with a high content of dietary protein and including fermented soybean cake, on the growth performance of organic pigs weaned outdoors. In total, 600 organic pigs weaned at d 49 on average were included in the study. The study comprised of five outdoor feeding trials lasting 46 d on average. The initial 14 d, pigs were fed an organic standard starter diet (control ($n = 5$); 134 g standardized ileal digestible crude protein/kg) using toasted soybean cake as the main protein source or high protein starter diet (HP ($n = 5$); 167 g standardized ileal digestible crude protein/kg) using fermented soybean cake as the main protein source. After 14 d the dietary differentiation ceased, and the pigs were offered a standard organic grower diet. Initial and final bodyweight and feed consumption were recorded at paddock level and diarrhea traits were visual assessed during the feeding trial. Pigs fed control starter diet had a 3% greater average daily feed intake compared to pigs fed HP starter diet ($P = 0.022$). Moreover, the average daily gain was increased by 8% ($P = 0.013$) and the feed conversion ratio was improved by 12% ($P = 0.001$) when pigs were fed the HP diet as compared to the control diet. The improved feed efficiency of pigs fed the HP diet, improved the calculated N efficiency and reduced the estimated N excretion per produced pig. In spite of the high protein content of the HP starter diet, the subjective diarrhea evaluation did not indicate increased risk of developing post-weaning diarrhea. In conclusion, organic pigs weaned outdoor benefitted from being fed a starter diet high in protein using fermented soybean cake as the major protein source. Growth performance was improved, without indication of increased risk of developing post-weaning diarrhea.

1. Introduction

Weaning is a critical period, when the young pig is exposed to a vast range of stress factors, it is immunologically vulnerable and is undergoing physiological development. The gastrointestinal tract of weaned pigs is challenged in the post-weaning period and the pigs are at risk of

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developing post-weaning diarrhea (PWD) (Pluske et al., 1997; Lalles et al., 2007). The PWD is a complex and multi-factorial disease, with nutrition as a key risk factor (Lalles et al., 2004). During the first two weeks post-weaning, pigs have a lower ability to digest and absorb amino acids (AA) (Engelsmann et al., 2022). Undigested protein passes into the hindgut, which increases proliferation of harmful bacteria and production of potentially toxic metabolites, increasing the risk of developing PWD (Williams et al., 2002; Gao et al., 2019).

European legislation requires a prolonged lactation period for organic sows and their piglets (Regulation (EU), 2018). The prolonged lactation period reduces the risk of PWD because the dietary change occurs more gradual, and the gastrointestinal tract is more developed at weaning as compared with conventional rearing practices. Nevertheless, PWD is a major health issue also in the European organic pig production (Leeb et al., 2014).

Composing feed for organic pigs is challenging, due to the limited range of feedstuffs available and the ban of certain feed additives, including crystalline AA (Regulation (EU), 2018). Typically, the AA composition of feedstuffs is suboptimal, and the concentrations of especially lysine, methionine, threonine and tryptophan are low compared to the requirement of the pig. The content of dietary crude protein (CP) in weaner diets are kept low, to reduce the risk of developing PWD (Lynegaard et al., 2021) and due to environmental concerns (Eriksen et al., 2002). However, diets low in protein may also reduce productivity (Opapeju et al., 2009).

Although the genetics of most organic pigs in Denmark are similar to the conventional pigs (Früh et al., 2014), the growth rate is lower than in conventional pig production, indicating that the growth potential of organically raised weaners is not fully explored (Hansen, 2020). Using high-quality protein sources with favorable AA profile and high digestibility in organic pig production is an approach for improving the growth performance of weaners. Fermented soybean products have a well-balanced AA profile and the content of anti-nutritional factors, such as trypsin inhibitors and oligosaccharides have been reduced in the fermentation process resulting in high content of digestible protein (Kim et al., 2006; Liu et al., 2021). Replacing toasted soybean products with fermented soybean products, has been shown to increase the growth performance, and reduce the risk of developing PWD in weaned pigs (Kiers et al., 2003; Wang et al., 2007; Liu et al., 2021).

This study hypothesized that feeding organic pigs weaned outdoor a starter diet high in protein based on fermented expeller-extruded soybean cake the initial 14 days after weaning would improve growth performance. Furthermore, we assumed the increased productivity to carry on after the dietary treatment ceased. Also, it was hypothesized that the increased content of dietary protein would not increase the risk of outdoor weaned pigs to develop PWD, due to the inclusion of fermented soybean cake.

2. Materials and method

The study was carried out from ultimo April 2019 to mid-February 2020 in a commercial organic pig herd located in the Western part of Denmark. The animal study was conducted in accordance with the Danish Ministry of Justice, Law 474 of 15. May 2014 and executive order 2028 of 14. December 2020 regarding animal experiments issued by the Danish Ministry of Environment and Food.

2.1. Experimental design, animals and housing

The study was designed as block feeding trial to test different starter diets (fed for 14 d) for organic pigs weaned outdoor. A total of 600 organic Duroc x [Danish Landrace x Yorkshire] crossbred pigs weaned at d 49 were included in the study. During lactation from d 21 of age all piglets were offered a standard organic starter diet. Feeding trials were initiated immediately after weaning at d 49 of age. For each of five blocks, 120 newly weaned pigs were randomly allocated across litters to one of two starter diets: A standard organic starter diet as fed during the lactation period (control, $n = 5$) or an organic high protein starter diet including fermented soybean cake (HP, $n = 5$). Pigs were allocated in one of two paddocks according to the dietary treatment. At d 14, all pigs changed to a standard organic grower diet. The feeding trials lasted 46 d on average. According to the organic regulations of the European Union, pigs were also supplied grass-clover silage ad libitum during the experimental period. The blocks were evenly distributed throughout the study period: Three blocks in the summer ($n = 6$) and two blocks in the winter ($n = 4$). For every second block, a new paddock area was employed. The paddocks measured 1020 m^2 (31 m x 33 m) and an isolated hut measuring 14.5 m^2 (5.6 m x 2.6 m) was located in each paddock.

The paddocks were sown with a commercial organic grass-clover mixture (ForageMax 56A, DLF Trifolium, Roskilde, Denmark), consisting of 10% Trifolium Repens (white clover; Rivendel), 30% Lolium Perenne (perennial ryegrass; Humbi 1) and 60% Festuca Rubra (red fescue; Gondolin). The proportion of white clover were increased to 16% to 18%, as additional white clover seed was added to the mixture.

During the study, pigs had free access to drinking water, and when the air temperature was above 15 °C the pigs were allowed the possibility for wallowing. The health status of the pigs was continuously monitored and if necessary, pigs were treated and removed from the study.

2.2. Diets and feeding

A commercially available organic starter diet, based on organic cereals and toasted expeller-extruded soybean cake, was used as control diet (Table 1). The control diet contained 8.79 MJ NE/kg, 134 g standardized ileal digestible (SID) CP/kg and 7.68 g SID lysine/kg (Table 2, calculated). The HP diet was based on organic cereals and fermented expeller-extruded soybean cake (FSBC) (Table 1). The soybean cake was soaked with water and inoculated with Bactocell® (*Pediococcus Acidilactici CNCM I-4622*), approved for organic production. The fermentation was carried out for 14 d, after which the fermentate was dried. The HP diet contained 9.01 MJ NE/kg, 167 g SID CP/kg and 9.96 g SID lysine/kg (Table 2, calculated). The grower diet fed after 14 d was based on organic cereals and toasted soybean cake (Table 1), and contained 8.83 MJ NE/kg, 154 g SID CP/kg and 8.19 g SID lysine/kg (Table 2, calculated). All diets were formulated to supply the recommended concentrations of vitamins and minerals, according to Danish nutrient standards (Tybirk et al., 2020).

The pigs were offered feed ad libitum during the feeding trial. The feed was supplied in a group feeding system, designed for outdoor pig production, with 18 feeding points and a capacity of 600 kg. At the end of each period feed residues were weighed. All diets were produced at a commercial feed factory (Vestjyllands Andel, Vildbjerg, Denmark).

2.3. Recording and sampling

The feed use was calculated from the feed provided and the residues collected at the end of each period, and thus includes potential feed wastage. The mean bodyweight (BW) of pigs were measured paddock-wise by the farmer, at the entry and at the end of the feeding trials in a drive-over weighing system based on four load cells placed on a firm and stable surface. The weighing system was calibrated by a trained technician at the beginning of the experimental period and by the farmer at the beginning and end of each feeding trial before weighing the pigs. Thus, all productivity parameters are expressed with paddock as the experimental unit. If pigs died or were removed during the study, the BW and date was noted by the farmer. Dead and removed pigs were pooled into one category before analyzed.

Four times during each period, the pigs were subjectively evaluated by trained technicians on three diarrhea traits at paddock level: Indicators of diarrhea, prevalence and severity of diarrhea (Table 3).

Table 1
Ingredients of organic experimental diets.

Ingredients, g/kg as-fed	Starter diets[1]		Grower diet[2]
	Control	High protein	
Barley/wheat/dehulled oat, gelatinized[3]	333	300	
Oat	250	150	150
Barley	170	229	350
Wheat			160
Oat flakes			50.0
Expeller-extruded soybean cake, fermented		200	
Expeller-extruded soybean cake, toasted	80.0		150
Fava beans			50.0
Fish meal	38.5	44.0	50.3
Starfish meal	30.0		
Dried yeast	25.0	12.5	
Peas	20.0		
Potato protein concentrate		6.3	
Coconut meal	6.3	6.3	
Seaweed meal	6.3	6.3	
Skimmed milk powder	6.3	6.4	
Fenugreek powder	0.5	0.5	
Monocalcium phosphate	8.3	8.9	10.2
Sodium chloride	3.1	4.0	4.2
Calcium carbonate	2.8	5.8	7.8
Vitamins and micro-minerals[4,5]	4.3	4.4	2.6
Feed additives[6]	16.3	16.3	15.6

[1] Offered in 14 days from the experimental start.
[2] Offered from d 14 to d 46 of the experiment (experimental end).
[3] A mixture of one third barley, one third wheat and one third dehulled oat.
[4] Per kilogram starter diet: 10,000 IU vitamin A, 2000 IU vitamin D_3, 200.0 mg vitamin E, 4.00 mg vitamin B_1, 12.0 mg vitamin B_2, 6.0 mg vitamin B_6, 0.05 mg vitamin B_{12}, 5.0 mg vitamin K_3, 35.0 mg D-pantothenic acid, 50.0 mg niacin, 0.20 mg biotin, 2.0 mg folic acid, 150.0 mg iron ($FeSO_4$), 2.0 mg iodine ($Ca(IO_3)_2$), 80.0 mg cupper ($Cu_2(OH)_3Cl$), 60.0 mg manganese (MnO), 100.0 mg Zink ($Zn_5(OH)_8Cl_2 \cdot H_2O$), 0.25 mg selenium ($Na_2SeO_3$).
[5] Per kilogram grower diet: 5500 IU vitamin A, 1100 IU vitamin D_3, 155.0 mg vitamin E, 2.20 mg vitamin B_1, 4.40 mg vitamin B_2, 3.30 mg vitamin B_6, 0.02 mg vitamin B_{12}, 4.40 mg vitamin K_3, 11.0 mg D-pantothenic acid, 22.0 mg niacin, 0.22 mg biotin, 165.0 mg iron ($FeSO_4$), 0.44 mg iodine ($Ca(IO_3)_2$), 130.0 mg cupper ($Cu_2(OH)_3Cl$), 44.0 mg manganese (MnO), 100.0 mg Zink ($Zn_5(OH)_8Cl_2 \cdot H_2O$), 0.30 mg selenium ($Na_2SeO_3$).
[6] A mixture of commercially available organic acids, pre- and probiotics, enzymes etc. to support animal gut health.

Temperature was recorded inside the huts during the experimental period.

2.4. Calculation and statistical analysis

The average daily feed intake (ADFI; kg/d) was calculated using the total feed use within the paddock, number of pigs and duration of feeding trials. The estimated feed consumption of removed pigs was deducted from the total feed use. It was assumed that pigs consumed 2 kg feed/kg BW gain from weaning until death or removal. The average daily intake of lysine (g SID lysine/d) was calculated using similar procedure.

The average daily gain (ADG; g/d) was calculated based on the total BW gain and number of pigs within the paddock and duration of feeding trials.

The feed conversion ratio (FCR; kg gain/kg feed) was calculated using ADFI and ADG. The lysine efficiency (kg gain/g SID lysine) was calculated using a similar procedure.

The N balance was calculated as N intake minus N retention for a standardized pig at weaning (15 kg) until 35 kg. Intake of N originated from both compound feed and roughage. The FCR and CP content in the diets were used to calculate the amount of N supplied by compound feed. It was assumed that the pigs on average consumed 34 g DM of roughage each day during the feeding trials (Christiansen, 2020). The CP content of the grass-clover silage was assumed to be 136 g/kg DM (Eskildsen et al., 2020; Johannsen et al., 2022). The N content was calculated as CP/6.25. The retention of N was calculated as 29.6 g N/kg BW gain (Børsting et al., 2020).

The following statistical model was used to analyze BW, ADFI, average daily lysine intake, ADG, FCR and lysine efficiency.

Table 2
Chemical composition of organic experimental diets as-fed (calculated content based on regular analyses of the feed components).

Chemical composition	Starter diets[1]		Grower diet[2]
	Control	High protein	
Dry matter, g/kg	894	900	879
FUgp/kg[3]	1.03	1.06	1.04
Net energy, MJ/kg[4]	8.79	9.01	8.83
Crude protein, g/kg	163.5	194.2	183.8
SID crude protein, g/kg[5]	134.2	167.2	154.1
Fat, g/kg	46.6	50.8	42.9
Ash, g/kg	55.5	53.9	54.9
Amino acids, g SID/kg[5]			
Lysine	7.68	9.96	8.52
Methionine	2.60	3.03	2.79
Cystine	2.39	2.80	2.55
Threonine	5.59	6.73	5.63
Tryptophan	1.72	2.14	1.89
Isoleucine	5.88	7.90	6.73
Leucine	10.2	13.1	11.3
Histidine	3.26	4.36	3.79
Phenylalanine	6.22	8.28	7.29
Tyrosine	4.59	6.15	5.21
Valine	7.03	9.03	7.83

[1] Offered in 14 days from the experimental start.
[2] Offered from d 14 to d 46 of the experiment (experimental end).
[3] Danish feed units for growing pigs (Tybirk et al., 2006). The Danish feed evaluation system is based on potential physiological energy, closely related to the net energy system (Patience, 2012).
[4] The content of net energy was calculated from the Danish feed units (FUgp) in accordance with Theil et al. (2020).
[5] Standardized ileal digestible.

Table 3
Ethogram with category, score and description of the investigated traits of diarrhea.

Category	Score	Description
Indicators of diarrhea	1 - Normal feces	No signs of diarrhea.
	2 - Mild diarrhea	One indicator of diarrhea in the paddock (*either* consistency, color *or* abnormal smell) *and* less than 25% of the feces are diarrhea.
	3 - Some diarrhea	One indicator of diarrhea in the paddock (*either* consistency, color *or* abnormal smell) *and* more than 25% of the feces are diarrhea.
	4 - Severe diarrhea	More indicators of diarrhea in the paddock (consistency, color *and/or* abnormal smell).
Prevalence of diarrhea	1 - No signs	No animal showing signs of diarrhea.
	2 - Diarrhea	Up until 3 animals showing indication of diarrhea.
	3 - Some diarrhea	4 to 10 animals showing indication of diarrhea.
	4 - Plenty diarrhea	11 to 30 animals showing indication of diarrhea.
	5 - Severe diarrhea	More than 50% of the animal showing indication of diarrhea.
Severity of diarrhea	1 - Not affected	Animals with diarrhea are not affected.
	2 - Affected	Up until 25% of the animals with diarrhea showing one or more sign of reduced health (e.g., long-haired, sunken flank, visible spine, drooping).
	3 - Severely affected	More than 25% of the animals with diarrhea showing sign of reduced health.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$$

Where Y_{ijk} is the observed trait, μ is the overall mean of observations, α_i is the effect of dietary treatment (i = control or HP), β_j is the seasonal effect (j = summer or winter) and ε_{ijk} is the residual variation following a normal distribution ($k = 1, \ldots, n_{ij}$) with n_{ij} being 6 for j = summer and 4 for j = winter.

Mortality and removal rate was analyzed using a Poisson regression model with a logarithm link function.

$$\log(\mathrm{E}[Y_{ij}]) = \log(N_{ij}) + \mu + \alpha_i$$

Where Y_{ij} is the number of pigs dying or being removed, μ is the overall log-mean, α_i is the effect of dietary treatment (i = control or HP) and N_{ij} is the total number of pigs for the j'th pen ($j = 1, \ldots, 5$).

To investigate the seasonal effect on mortality and removal rate, a similar model was used, however α_i is the seasonal effect (i = summer or winter) and N_{ij} is the total number of pigs for the j'th pen ($j = 1, \ldots, n_i$) with n_i being 6 for i = summer and 4 for i = winter.

A two-sided Fisher's exact test was used to determine if there was a significant association between indicators, prevalence and severity of diarrhea and dietary treatment or season.

Statistical analyses were performed using the statistical software R (R Core Team, 2020). The package emmeans (Lenth, 2021) was used to analyze the effects of the experimental factors and obtain values of LSmeans. Effects were considered significant when the *P*-value was below 0.05.

3. Results

During the study, 33 pigs died or were removed due to health issues (20 control and 13 HP). The average outdoor temperature was 12.8 °C in the summer and 5 °C in the winter according to the local weather station (DMI, 2021). The average temperature inside the huts was 20.6 °C and 14.3 °C during summer and winter, respectively.

The FSBC had a CP content of 422 g/kg and a lysine content of 25.4 g/kg, with a standardized ileal digestibility of 93.0% and 98.6%, respectively. The content of SID CP was 392.5 g/kg and the content of SID lysine was 25.1 g/kg.

3.1. Growth performance

In Table 4, the effect of dietary treatment and season on BW, ADFI, CP and lysine intake, ADG, FCR and lysine efficiency of the weaned pigs are presented. The final BW, ADFI, ADG, FCR and lysine efficiency were affected by dietary treatment. Pigs fed the HP diet had an ADFI of 0.99 kg/d, while pigs fed the control diet had an ADFI of 1.02 kg/d (P = 0.022). Pigs fed the HP starter diet had a greater ADG than pigs fed the control starter diet (500 g/d vs. 464 g/d, P = 0.013). Consequently, HP pigs weighed 36.4 kg, while control pigs weighed 34.9 kg at the end of the feeding trials (P = 0.015). The FCR was 0.51 kg gain/kg feed for HP pigs and 0.45 kg gain/kg feed for control pigs (P = 0.001). The lysine efficiency was 0.058 kg gain/g SID lysine and 0.054 kg gain/g SID lysine for HP and control pigs, respectively (P = 0.029).

The initial BW of pigs, ADG and lysine efficiency were affected by season. In the summer, the newly weaned pigs were heavier compared to winter (14.1 kg vs. 13.1 kg, P = 0.015). During winter, pigs had an ADG of 500 g/d and this decreased to 464 g/d in the summer (P = 0.014). Moreover, the lysine was used more efficiently in the winter compared to summer (0.057 kg gain/g SID lysine vs. 0.055 kg gain/g SID lysine, P = 0.049).

3.2. Nitrogen balance

In Table 5 the calculated N balance of a standardized organic pig weaned outdoor (growing from 15 to 35 kg) is presented. The only input that differed between the groups was the input of N from compound feed: 1.27 kg N for control pigs and 1.17 kg N for HP pigs, per pig produced. Consequently, the excretion via feces and urine surplus was estimated to 0.72 kg N and 0.62 kg N for control and HP pigs, respectively. Thus, the N efficiency was calculated to 45% for pigs fed control starter diet and 49% for pigs fed HP starter diet.

3.3. Mortality and diarrhea parameters

According to the analysis of variance on the Poisson models no difference in mortality and removal rate was found between dietary treatments (P = 0.22) or season (P = 0.43).

In Table 6, the distribution and frequency of the subjective score of indicators, prevalence and severity of diarrhea, respectively, between dietary treatment and season are presented. Moreover, the results of Fisher's exact test are shown. According to Fisher's exact test, there was no association between indicators, prevalence and severity of diarrhea and dietary treatment. A statistically association between prevalence of diarrhea and season was found ($P < 0.001$), indicating a greater prevalence of diarrhea among weaned pigs during summer compared to the winter.

Table 5
Calculated N balance at animal level for a standardized (growing from 15 kg to 35 kg) organic pig weaned outdoor fed control or high protein starter diet, expressed as kilogram N per standardized pig produced.

Item	Control	High protein
Compound feed, kg N/pig	1.27	1.17
Roughage, kg N/pig	0.04	0.04
Retention, kg N/pig	−0.59	−0.59
Excretion, kg N/pig	0.72	0.62
N efficiency,%	45	49

Table 4
Effect of dietary treatment and season on bodyweight (BW), average daily gain (ADG), average daily feed intake (ADFI), crude protein (CP) and standardized ileal digestible (SID) lysine intake, feed conversion ratio (FCR) and lysine efficiency of organic pigs weaned outdoor and fed control or high protein starter diets.

Item	Dietary treatment[1]			Season[2]			*P*-value	
	Control	High protein	SEM	Summer	Winter	SEM	Diet	Season
Initial BW, kg	13.7	13.5	0.21	14.1	13.1	0.23	0.66	0.015
Final BW, kg	34.9	36.4	0.34	35.6	35.7	0.38	0.015	0.86
ADFI, kg/d	1.02	0.99	0.01	0.99	1.02	0.01	0.022	0.078
CP intake, g/d	184	183	1.55	181	186	1.72	0.89	0.075
Lysine intake, g SID lysine/d	8.53	8.68	0.07	8.50	8.71	0.07	0.16	0.073
ADG, g/d	464	500	7.8	464	500	8.7	0.013	0.014
FCR, kg gain/kg feed	0.45	0.51	0.01	0.47	0.49	0.01	0.001	0.053
Lysine efficiency, kg gain/g SID lysine	0.054	0.058	0.001	0.055	0.057	0.001	0.029	0.049

[1] Control (n = 5), High protein (n = 5).
[2] Summer (n = 6), Winter (n = 4).

Table 6
Distribution and frequency of subjective scores between dietary treatment and season of indicators, prevalence and severity of diarrhea among organic pigs weaned outdoor and fed control or high protein starter diets. P-values are from Fisher's exact test.

Item	Dietary treatment[1]			Season[2]		
	Control	High protein	*P*-value	Summer	Winter	*P*-value
Indicators of diarrhea			0.72			1.00
Normal feces	7 (47%)	8 (53%)		11 (50%)	4 (50%)	
Mild diarrhea	8 (53%)	6 (40%)		10 (45%)	4 (50%)	
Some diarrhea	0	1 (7%)		1 (5%)	0	
Severe diarrhea	0	0		0	0	
Prevalence of diarrhea			0.80			<0.001
No signs	1 (5%)	1 (5%)		1 (4%)	1 (7%)	
Diarrhea	10 (50%)	11 (58%)		7 (29%)	14 (93%)	
Some diarrhea	8 (40%)	5 (26%)		13 (54%)	0	
Plenty diarrhea	1 (5%)	2 (11%)		3 (13%)	0	
Severe diarrhea	0	0		0	0	
Severity of diarrhea			0.54			0.14
Not affected	17 (89%)	15 (79%)		18 (75%)	14 (100%)	
Affected	2 (11%)	2 (11%)		4 (17%)	0	
Severely affected	0	2 (11%)		2 (8%)	0	

[1] Control ($n = 5$), High protein ($n = 5$).
[2] Summer ($n = 6$), Winter ($n = 4$).

4. Discussion

4.1. Effect on productivity

Feeding organic pigs weaned outdoor the HP starter diet improved growth performance. The ADFI of control pigs was 3% greater compared to HP pigs, however pigs fed the HP starter diet had an 8% greater ADG and 12% more efficient FCR. Also, lysine was used more efficiently when pigs were fed the HP starter diet as compared to the control starter diet.

In the current study, the HP diet was composed using FSBC at a high concentration. Fermented soybean products have a higher content of CP and AA compared to traditional soybean products (Cervantes-Pahm and Stein, 2010; Kim et al., 2015). The current study was conducted on organic pigs and thus, expeller-extruded soybean cake was used as protein source. In the conventional pig production typically solvent extracted soybean meal is used. Soybean meal (SBM) has a lower fat content and a higher CP content as compared to soybean cake (Moura et al., 2015). The FSBC used in the current study had a SID CP content of 392.5 g/kg and a SID lysine content of 25.1 g/kg. Expectedly, this is slightly lower compared to fermented SBM used in other studies (Zhang et al., 2013; Upadhaya and Kim, 2015; Espinosa et al., 2020). However, the calculated standardized ileal digestibility of the FSBC was very high compared to the general findings on fermented SBM (Pedersen et al., 2016). The SID CP content was 25% greater and the SID lysine content was 30% greater in the HP diet as compared with the control diet. Due to the experimental diet compositions, it was not possible to identify whether the treatment effects were derived by the properties of FSBC (e.g. antinutritional factors) or the greater levels of dietary protein and AA in general. Individually, both factors have proved to affect the performance of weaned pigs.

Dietary protein affects the growth performance of weaned pigs (Wellock et al., 2006; Opapeju et al., 2009; Sloth et al., 2017; Lynegaard et al., 2021): An increased dietary concentration of CP improves ADG and FCR. Sloth et al. (2017) found that increasing the dietary content of SID lysine from 8.8 g/kg to 10.7 g/kg in diets for conventional produced pigs (16 to 31 kg) improved the ADG and FCR by 11% and 9%, respectively. These results agree with our findings. However, in the current study, the pigs were only fed the experimental diet for 14 d, while the pigs were fed experimental diets during the entire feeding trial in the study by Sloth et al. (2017). Thus, it seems that the pigs fed the HP starter diet continue to grow better and more efficiently during the remaining of the feeding trial, as compared with control pigs.

Feeding weaned pigs fermented soybean products has previously been shown to improve growth performance, as compared to toasted soybean products (Kiers et al., 2003; Wang et al., 2007; Liu et al., 2021). Fermentation of soybean products has been found to increase the ileal digestibility of CP and AA, due to chemical changes occurring during the processing (Upadhaya and Kim, 2015; Jeong et al., 2016). Firstly, the content of trypsin inhibitor is reduced (Hong et al., 2004; Cervantes-Pahm and Stein, 2010; Jeong et al., 2016). Secondly, large peptides undergo breakdown during fermentation (Hong et al., 2004; Jeong et al., 2016). This includes the antigenic proteins; glycinin and β-conglycinin, which are poorly digested by pigs (Zhao et al., 2015). And thirdly, fermentation reduces the content of the indigestible oligosaccharides raffinose and stachyose (Cervantes-Pahm and Stein, 2010; Liu et al., 2021). In the current study, the content of CP was 19% greater, in the HP diet compared to the control diet, however, the content of SID CP was 25% greater. Thus, the digestibility of CP has been increased, presumable due to the inclusion of FSBC, which had a high digestibility of CP and AA. This is in agreement with previous studies (Upadhaya and Kim, 2015; Jeong et al., 2016). However, not all studies find improved ileal digestibility of CP and AA when SBM is fermented (Pedersen et al., 2016): During the additional processing of fermented soybean products there is a risk of overheating and forming Maillard products, especially reducing the lysine digestibility.

The concentration of SID lysine in the control and HP starter diet was 71% and 89% of the recommended level, respectively, and the concentration of SID methionine was 74% and 84% of the recommended level, respectively, according to Danish nutrient standards (Tybirk et al., 2020). Thus, lysine was the first limiting AA in the control diet and methionine appeared to be the first limiting AA in the HP diet. In future studies, adjusting the AA composition should be considered, e.g. by using two high-quality protein sources with different AA profiles, to optimize the use of the essential AA.

In the current study, the performance of pigs fed the control starter diet was slightly below the average productivity of Danish commercial organic herds, while pigs fed HP starter diet performed almost similar to the average (Christiansen, 2020). In Denmark, organic piglets are born and reared outdoors and are typically weaned into indoor straw-bedded pens with access to an outdoor concrete pen, and this should be taken into consideration when comparing the results. Pigs reared outdoor often have a greater level of physical activity and a higher energy requirement for thermoregulation. Consequently, Gentry et al. (2004) found that the ADFI is greater and FCR is lower for pigs reared outdoors, as compared with pigs reared indoors. When reared under optimal production conditions and fed according to the Danish nutrient recommendations (11.8 g SID lysine/kg) the ADG and FCR of indoor weaned pigs (16 to 31 kg) were found to be 713 g/d and 0.63 kg gain/kg feed (Sloth et al., 2017). This is considerably better (43% and 20%, respectively) compared to the current study and indicates that the growth potential is not fully exploited in organic pigs. Certain obstacles prevent organic producers to fulfill the potential of the pigs, especially the ban on crystalline AA (Regulation (EU), 2018). Without crystalline AA the ratio between CP and AA cannot be properly optimized to the requirement of the pig. In organic diets, the CP content typically is high relative to the first limiting AA, but feeding excess protein is energetically unfavorable for the animal and decreases the feed efficiency (Le Bellego et al., 2001).

During winter conditions the growth performance of organic pigs weaned outdoor increased. In the winter feeding trials pigs had on average 8% greater ADG compared to pigs fed in the summer. Moreover, lysine was used more efficiently, the ADFI tended to be greater and FCR tended to be improved in the winter periods. Typically, the feed intake is greater during winter as the pig require a higher amount of energy for thermoregulation (Honeyman and Harmon, 2003). This reduces the feed efficiency; however, such reduction was not observed in the current study. Possibly, pigs had a low physical activity during winter and rested inside the huts and thereby reduced their heat production. This is supported by Kongsted et al. (2013), who registered outdoor finisher pigs rested inside their huts 63% to 79% of the observations, during winter conditions. In the current study, the temperature inside the huts only went below the lower critical temperature (6 °C to 11 °C depending on BW) of weaning pigs with straw bedding, a few nights during the winter (Bruce and Clark, 1979). Consequently, the requirement for thermoregulation was most likely not elevated when they were inside the huts. Moreover, it was a relative mild winter with an average outdoor temperature of 5 °C.

4.2. Nitrogen balance

Weaning organic pigs outdoor are associated with risk of a high N loss, especially in terms of N leaching from the paddocks (Halberg et al., 2010). In the current study we estimated that organic pigs weaned outdoor fed the HP starter diet had improved N balance and N efficiency. The improved FCR of the HP diet fed pigs reduced the estimated intake of N from compound feed, in spite of the greater CP content of the HP starter diet. Thus, according to our calculations the environmental impact of organic pigs weaned outdoor is reduced at the animal level when fed the HP starter diet.

In conventional pig production systems, a lower N excretion and greater N efficiency is typically observed as compared with organic production systems. Applying the results of Lynegaard et al. (2021) on conventional weaning pigs into an N balance, an estimated efficiency of 67% was achieved with an excretion of only 0.29 kg per produced pig (growing from 15 kg to 35 kg). This is 53% less than the calculated N excretion of the pigs fed the HP diet in the current study. The high efficiency and low excretion are achieved as a result of a better FCR and a low dietary content of CP. This underlines the importance of improving the FCR, by optimizing the dietary composition, especially the content of CP and the AA profile, in order to minimize the environmental impact.

4.3. Effect on post-weaning diarrhea

The subjective evaluation of diarrhea traits showed no indication that feeding organic pigs weaned outdoor the HP starter diet increased prevalence or severity of PWD. It should be kept in mind that visual assessment of diarrhea traits under outdoor conditions are associated with a high degree of uncertainty: The area and number of pigs are large, and the pigs avoid humans. Moreover, the weather constitutes a considerable source of error.

It is well-known that increased dietary protein increases the risk of PWD (Opapeju et al., 2009; Lynegaard et al., 2021), however in the current study, the inclusion of FSBC seems to have counteracted this effect. Literature suggests, that replacing traditional soybean products with fermented products, reduces the risk of PWD (Kiers et al., 2003; Wang et al., 2007; Liu et al., 2021). Different mechanisms for the beneficial effect of fermented soybean products on PWD are proposed. First, the higher digestibility of fermented soybean products reduces the amount of undigested protein reaching the hindgut. Excessive amounts of undigested protein in the hindgut promotes proliferation of pathogenic bacteria and the production of toxic metabolites, such as ammonia, amines and sulfide hydroxide, inducing diarrhea (Gao et al., 2019). Moreover, fermented soybean products are believed to have probiotic properties (Wang et al., 2007), and SBM fermented by *Lactobacilli* has been found to reduce the content of coliform bacteria through the gastrointestinal tract of weaned pigs (van Winsen et al., 2001). The authors suggest the increased concentration of lactic acid, reduced nutrient availability for the pathogens, and competition for receptor sites are likely explanations of this beneficial effect.

The piglet's ability to digest CP and AA is very low during the first two weeks post-weaning and time after weaning is more important for protein digestibility than the source of protein. Fewer mature epithelial cells and less absorptive area in the early post-weaning period may partly explain the poor protein digestibility (Engelsmann et al., 2022). The villus height is shortened, and crypt depth is reduced, which impairs the digestive and absorptive function of the small intestine and may contribute to development of PWD (Heo et al., 2013). Wang et al. (2007) and Liu et al. (2021) found that feeding newly weaned pigs a fermented soybean product increased the villus height and crypt depth. The reduction of the antigenic proteins; glycinin and β-conglycinin during fermentation, may explain this effect, because these proteins are known to cause damage to the intestinal epithelium (Zhao et al., 2015)

5. Conclusion

Organic pigs weaned outdoor benefitted from being fed a HP starter diet. The 25% greater content of SID CP and corresponding higher concentrations of essential AA improved the ADG and feed efficiency by 8% and 12%, respectively, compared with pigs fed control starter diet. The dietary content of SID CP and AA composition was improved by using FSBC as the primary protein source in the HP diet. The FSBC presumably contributed to the improved growth performance of weaned pigs, due to the improved nutritional value. The calculated N balance of pigs fed HP starter was improved, as a result of the greater feed efficiency, which in turn reduced the estimated environmental impact. At last, we found no indication that the risk of developing PWD was affected by the dietary treatments, despite the high CP content of the HP diet. The high CP digestibility and probiotic properties of FSBC, most likely counterbalanced the high CP content regarding the risk of PWD.

Declaration of Competing Interest

All authors declare that they have no conflicts of interests.

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