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Performance and diarrhoea in piglets following weaning at seven weeks of age: Challenge with *E. coli* O 149 and effect of dietary factors

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ABSTRACT

Four dietary factors (*ad libitum* versus feed restriction, control versus protein restriction at *ad libitum* feeding, control versus inclusion of lupin as a protein source at *ad libitum* feeding, and control versus extra vitamin E at *ad libitum* feeding) were tested in four separate experiments for the effect on diarrhoea. To introduce a diarrhoea-like condition, half of the piglets were challenged with an *E. coli* O 149 dose of 1×10^8 colony forming units on days one and two after weaning (day of weaning = day zero). All piglets were susceptible since the dams were tested mono-zygotic susceptible to the attachment site of *E. coli* O 149 in the intestines. Each of the four experiments included 32 piglets from 4 sows. The design was a 2×2 factorial with dietary factor and *E. coli* O 149 challenge as the two factors, each at two levels. The piglets were housed individually during the experiment which lasted for 10 days from weaning at 7 weeks of age. The daily recordings included feed intake, weight and faecal score (from 1 = solid and cloddy to 6 = watery and yellow). Faeces from days 1 to 4 were tested for *E. coli* strains. In addition, blood was sampled and serum was analysed for antibodies to *E. coli*, IgG and IgM. Generally the *E. coli* challenge had no effect on growth and feed intake whereas faecal score and number of faecal haemolytic bacteria increased and faecal dry matter decreased. Feed restriction decreased the weight gain while faecal characteristics were unaffected. An analysis including all four experiments revealed that a feed intake of less than 200 g during the first day after weaning seems to be associated with a relatively high incidence of a post-weaning diarrhoea-like condition. Protein restriction decreased faecal score and increased faecal dry matter while weight gain tended to decrease. Inclusion of lupin affected neither weight gain nor faecal characteristics. Extra vitamin E did not affect weight gain while faecal dry matter decreased, and faecal score and number of faecal haemolytic bacteria increased. The dietary treatments had no effect on the measured immunoglobulins. In conclusion, the studied dietary factors could not alleviate a diarrhoea-like condition and at the same time maintain the growth rate. Furthermore, the results indicate that performance can be improved if piglets achieve a daily feed intake of at least 200 g during the first day after weaning.

1. Introduction

Many piglets have a poor and variable growth rate associated with a low and variable feed intake after weaning. Furthermore, piglets have an increased susceptibility to enteric pathogens that may cause diseases among which "weaning diarrhoea" is the most common. Weaning diarrhoea usually occurs after a 3–4-day latency period and peaks around one week after weaning. Weaning diarrhoea is a multifactorial problem, and the clinical symptoms may be linked to a combination of different factors such as low feed intake during the first week after weaning, low hygiene, insufficient ventilation, low age at weaning, low piglet live weight at weaning, and a high number of piglets per pen (Madec et al., 1998).

Weaning diarrhoea is also a problem in organic pig production (S. Bak, personal communication) although organically produced piglets are relatively old at weaning

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(at least seven weeks in Denmark). Due to restrictions in the use of medication in organic pig production, other tools that may reduce weaning diarrhoea are needed. Therefore this study will focus on four dietary-based tools, i.e. feed restriction, protein restriction, inclusion of lupin in the diet and inclusion of extra vitamin E in the diet.

The matter of feed restriction is controversial since low feed intake may cause intestinal malfunction and damage (Spreeuwenberg et al., 2001; McCracken et al., 1999), but despite this, restricted feeding is commonly used as an approach to reduce weaning diarrhoea in Denmark (Jørgensen et al., 2000) and elsewhere (Lainea et al., 2004). Potentially, restrictive feeding may prevent piglets, which have not eaten significant amounts during the first one to two stressful days, to engorge on the weaning diet if it is available ad-lib. An engorgement might lead to digestive upset. Also the optimal level of dietary protein at weaning is somewhat controversial. Low-protein diets are commonly used to reduce weaning diarrhoea (Callesen, 2004), and have been shown to reduce the frequency of diarrhoea, however, at the expense of growth performance (Eggum et al., 1987).

Organic pig production is subject to regulations regarding sources of feedstuffs which results in great interest in testing protein sources that may be an alternative to non-organically grown soya beans. One of these alternatives may be lupin which is readily available and although low in sulphur-rich amino acids has a relatively high protein content. In addition lupin may have the potential to reduce intestinal *E. coli*, since it is rich in galactose, a substrate for galactane, which has been shown to reduce the number of intestinal *E. coli* (Mathew et al., 1993).

Vitamin E is important for development of and maturation of the immune system and vitamin E deficiency has been found to predispose pigs to *E. coli* infection (Ellis and Vorhies, 1976), which may lead to weaning diarrhoea, whereas dietary supplementation with vitamin E resulted in improved cellular and humoral immunity in pigs (Jensen et al., 1988; Hayak et al., 1989).

The effects of dietary factors on spontaneous weaning diarrhoea are difficult to study because of low or variable incidences of this disease. Therefore controlled *E. coli* challenge models have been used in order to simulate the outbreak of this condition (Madec et al., 2000; Melin et al., 2000). The experimental models of porcine post-weaning *colibacillosis* have used a combination of different strains for piglet inoculation (Madec et al., 2000) as well as a single pathogen strain (Melin et al., 2000).

The objective of this study was to determine the effect of four selected dietary factors on a potential reduction in severity of weaning diarrhoea in piglets, which were weaned at 7 weeks of age to simulate this condition in organic pig production.

2. Materials and methods

2.1. Animals

The piglets were from the herd at the Research Centre Foulum. The herd has the specific-pathogen-free (SPF) health status according to the Danish SPF system (i.e. free from toxigenic *Pasteurella multocida*, *Sarcoptes scabei* var. *suis*, *Haematopinus suis*, *Brachyspira hyodysenteria*, and *Actinobacillus pleuropneumoniae* serotype 1,2,3,4,5,7,8,9,10, but reinfected with *Mycoplasma hyopneumoniae*). The herd is not organic, but age at weaning met the requirements for organic pig production. Only sows tested homozygote carriers of the dominant gene encoding for intestinal F4 fimbriae receptors (Jørgensen et al., 2004) were used as dams while the sires were not tested. Regardless of genotype, however, the density of intestinal receptors for *E. coli* F4 adhesion is variable (Rasschaerta et al., 2007). The sows were not vaccinated against *E. coli*. However, *E. coli* vaccination is permitted in organic pig production. The piglets were weaned at 7 weeks of age to simulate this condition in organic pig production.

Piglets were treated for diarrhoea if they had a faecal score of 5 or more (see below) and appeared apathetic and not interested in their surroundings.

2.2. Experimental design

Four separate experiments were conducted each testing the effect of one of the four dietary factors, i.e. feed restriction, protein restriction, inclusion of lupine in the diet and inclusion of extra vitamin E in the diet. Each experiment was designed as a 2×2 factorial block design with dietary factor (two levels) and challenge with *E. coli* (inoculation with *E. coli* suspension or buffer) as the factors. The housing facility allowed handling of one block of 16 piglets at each time. Each block consisted of eight littermate piglets from each of two sows. For each dietary factor two blocks were used. Two piglets were allocated to each of the four factorial subgroups within each litter. Thus for each dietary factor, 32 piglets originating from four sows and allocated to the four subgroups in two blocks were used. During allocation of the piglets their weights were taken into consideration in order to control weight variation between subgroups. The experimental period was 10 days.

2.3. Dietary factors

The feed used in the feed restriction experiment was obtained from a commercial feed supplier while the other diets were produced at Research Centre Foulum. The composition of the diets is outlined in Table 1. The feed ingredients met the requirements for organic pig production.

2.3.1. Feed restriction

The control piglets had *ad libitum* access to the feed while the experimental piglets were fed restrictively starting with a daily allowance of 400 g feed on day 1 (first 24 h), gradually increasing by 40 g per day to 800 g feed on day 10 of the experiment. Half the daily restricted ration was fed in the morning and half in the afternoon. The feed used in this experiment was a commercial diet, thus the composition deviates from the control diet used in the other experiments.

2.3.2. Protein restriction

The dietary content of barley was increased at the expense of the protein rich ingredients, thus the control diet included 167 g (20.1% crude protein), while the low-protein diet included 94 g (12.0% crude protein) digestible protein per kg.

Table 1
Composition of the experimental diets.

Ingredients	Feed formulations				
	Feed restriction[a]	Control[b]	Protein restriction[c]	Lupin inclusion[d]	Extra Vitamin E[e]
Barley, organic, %	20.0	29.1	62.9	29.0	29.0
Oats, organic, %	6.6	12.0	12.0	12.0	12.0
Wheat, organic, %	31.0	15.0	15.0	15.0	15.0
Wheat, %	13.6	–	–	–	–
Fishmeal, %	13.8	–	–	–	–
Soya beans, toasted, organic, %	3.7	13.0	2.6	13.0	13.0
Sunflower cake, dehulled, %	3.0	–	–	–	–
Rape seed cake, double low, %	–	7.5	1.5	2.5	7.5
Peas, organic, %	6.0	15.0	3.0	5.0	15.0
Potato protein concentrate, %	–	6.0	1.2	6.0	6.0
Lupin, *L. angustifolius*, organic, %	–	–	–	15.0	–
Monocalcium phosphate, %	0.60	0.56	0.42	0.53	0.56
Calcium carbonate, %	1.13	1.28	0.82	1.38	1.28
Salt (NaCl), %	0.19	0.39	0.36	0.39	0.39
Vitamin mineral mix[f], %	0.40	0.20	0.20	0.20	0.20
Vitamin E[g], %	–	–	–	–	0.0625
MJ NE per kg	8.53	8.32	7.73	8.33	8.31
Digestible protein, g/kg	182	167	94	177	167

[a]Used for both the control and the feed restricted group in the experiment with feed restriction. Used as creep feed in both blocks.
[b, c]Used in the experiment with protein restriction including. Each diet used as creep feed in one block.
[b, d]Used in the experiment with lupine inclusion including use as creep feed. Each diet used as creep feed in one block.
[b, e]Used in the experiment with extra vitamin E including use as creep feed. Each diet used as creep feed in one block.
[f]I.E. per gram, mg per kg. *Feed restriction diet*: Vit. A 2200 I.E., vit. D3 500 I.E., α-tocopherol 30,000 mg, K3 vit. 1100 mg, B1 vit. 1100 mg, B2 vit. 2000 mg, B6 vit. 1650 mg, B12 vit. 11 mg, D-pantothenic acid 5500 mg, Niacin 11,000 mg, Biotin 27.5 mg, Fe (II) sulphate 25000 mg, Zn oxide 40,000 mg, Mn (II) oxide 13,860 mg, Cu (II) sulphate 10,000 mg, I (potassium iodide) 99 mg, Se (sodium selenite) 150 mg. *Other diets*: Vit. A 2500 I.E., vit. D3 300 I.E., α-tocopherol 33,000 mg, K3 vit. 2200 mg, B1 vit. 1100 mg, B2 vit. 1100 mg, B6 vit. 1650 mg, B12 vit. 11 mg, D-pantothenic acid 5500 mg, Niacin 11,000 mg, Biotin 27.5 mg, Fe (II) sulphate 44,000 mg, Zn oxide 55,000 mg, Mn (II) oxide 22,000 mg, Cu (II) sulphate 12,500 mg, I (calcium iodate) 114 mg, Se (sodium selenite) 175 mg.
[g]Natur E granulate, 40% (400,000 IU/kg = 300,000 mg RRR-α-tocopheryl acetate/kg), Pharmalett A/S, Kolding, Denmark.

2.3.3. Lupin inclusion

L. angustifolius was included at the expense of peas and rape seed cake.

2.3.4. Extra vitamin E

The basal diet contained 60 mg/kg of *all-rac*-α-tocopheryl acetate. The basal diet was supplemented with additional 150 mg/kg RRR-α-tocopheryl acetate.

2.4. Challenge with E. coli

The *E. coli* strain 9910045-1 (O149:F4) was used (named *E. coli* O 149 from here on). The *E. coli* O 149 was originally isolated at the Danish Institute for Food and Veterinary Research from the intestinal contents of a pig with weaning diarrhoea. According to polymerase chain reaction (PCR) analysis of virulence factor genes, the bacterial strain harbours genes for enterotoxins STb, LT, EAST1 and fimbriae F4ac (Frydendahl et al., 2001). *E. coli* O 149 causes beta-haemolysis when grown on blood agar (BA) (Colombia agar [Oxoid] supplemented with 5% calf blood).

E. coli O 149 was stored at −80 °C in a Luria-Bertani (LB) medium (Merck) with glycerol (1:1 v/v). For each inoculation, a fresh culture was prepared. Frozen *E. coli* O 149 was streaked on BA and grown at 37 °C for 18 h. A swab of colony material was suspended in 200 ml Veal Infusion broth (Merck) and grown for 5 h at 37 °C in an incubator with constant shaking at 200 rpm. After incubation the suspension was centrifuged (17,700 g) at 4 °C for 20 min. The bacterial pellet was resuspended in sterile 10% sodium chloride (NaCl). This bacterial suspension was diluted in serial ten-fold dilutions with NaCl as the diluent and plated on BA for quantitative determination of the *E. coli* colony forming units (CFU). Each piglet received an *E. coli* O 149 dose of 1×10^8 CFU in 20 ml NaCl on days 1 (day of weaning = day 0) and 2 (and 3 for the first experiment which was with feed restriction) via an oro-gastric tube. After the first experiment, it was our experience that piglets receiving *E. coli* on three consecutive days became too unthrifty for experimental purposes and thus *E. coli* inoculations on day 3 was omitted thereafter. Piglets destined to receive an *E. coli* O 149 dose that had a faecal score of 5 or more (see below), received sodium bicarbonate instead of the *E. coli* O 149 dose. Thus a total of ten piglets received one inoculation less than maximum planned. The oro-gastric tube was inserted and then gently flushed with a few ml of a buffer (sodium bicarbonate) to ascertain that the tube was correctly placed since buffer from a tube incorrectly placed in the lungs would make the pig cough. After inoculation the tube was flushed with approximately 30 ml sodium bicarbonate to ascertain that all suspension of *E. coli* was given to the piglet. Non-inoculated piglets received equivalent amounts of sodium bicarbonate via an oro-gastric tube.

All procedures involving animals were approved by the Danish Animal Experiments Inspectorate under the Ministry of Justice.

2.5. Feeding and housing

The sows were fed *ad libitum* during lactation. During the nursing period, the piglets had *ad libitum* access to creep feed from 14 days of age and accumulated consumption for each litter was recorded from 28 days of age. If waste occurred an estimate was subtracted from the consumption. Based on this recording and litter size, an estimated average daily creep feed uptake per piglet was calculated. The composition of the creep feed is outlined in Table 1. During the 10 day experimental period all piglets were fed *ad libitum*, except the restrictively fed piglets in the experiment on feed restriction. The piglets had free access to water.

At weaning piglets were moved to individual 0.8×1.3 m pens with concrete flooring and sawdust bedding. The piglets inoculated with *E. coli* were housed in one room, and the non-inoculated piglets were housed in an adjacent and similar

room in order to avoid cross contamination from the bacterial challenge. The non-inoculated animals were always handled before the *E. coli* inoculated animals. Environmental conditions such as temperature, air change and bedding were the same in the two rooms.

2.6. Daily recordings

Daily recordings were obtained for weight, feed intake and faecal score (1 = solid and cloddy, 2 = compact, 3 = soft with shape, 4 = soft and liquid, 5 = watery and dark, 6 = watery and yellow). The faecal scorings were performed by two persons jointly and during the experiments a total of three persons were involved.

2.7. Faecal sampling and analyses

On days 1, 2 and 3 of the experiment, faeces were collected from the rectum of the piglets. The day 1 sample was taken before inoculation with the *E. coli* O 149. Faeces were analysed for dry matter content (Association of Official Analytical Chemists, 1980) and for content of haemolytic *E. coli* according to the following bacteriological examination. A minimum of 1 g faeces was suspended in 10% NaCl solution (10 ml per g faeces) and homogenised by stomaching (BagMixer^R 400, Interscience, St. Nom, France). Quantification of the haemolytic *E. coli* faecal shedding was performed on serial ten-fold dilutions on BA (incubated at 37 °C for 18 h), with a detection range of 10^5 to 10^{11} Colony Forming Units (CFU) of Haemolytic bacteria per g faeces. When more than 50% of the colonies were haemolytic *E. coli*, 5 colonies were selected and sent to the Danish Institute for Food and Veterinary Research, where they were tested by seroagglutination for O 149 type according to Frydendahl (2002).

2.8. Blood sampling and analyses

Blood was obtained by puncture from the jugular vein day 0 and 8 from one block from the feed restriction, protein restriction and lupin inclusion experiments. Serum was obtained after centrifugation at 3000 ×g and stored at −80 °C until analyses. Serum concentrations of immunoglobulin G and M (IgG and IgM, respectively) were measured using commercial kits (pig ELISA quantification kit, Bethyl Laboratories, Montgomery, Texas). Measurements of antibodies specific to *E. coli* O 149 K88 in serum were done by indirect ELISA as described by Lauridsen and Jensen (2005). The titre values are reported as arbitrary values (i.e., the last dilution [$\times 10^2$] that gave a positive reaction).

2.9. Statistical analyses

All data from four piglets that died during the experiments were excluded from the analyses. All data from the piglets that received one inoculation less than their subgroup mates were included in the analyses. The performance and faecal data (see

Table 2
Performance of piglets weaned at seven weeks of age and allocated to different dietary treatments in combination with *E. coli* inoculation (LS means).

Treatment	1		2		SE[a]	*P*-value		
E. coli	−	+	−	+		Treatment	*E. coli*	Interaction
Feed restriction (1 ad libitum, 2 restricted)								
Feed intake, g/d	865	729	563	546	59	<0.001	0.12	0.22
Gain, g/d	475	340	244	275	57	0.004	0.28	0.09
Faecal score[b]	3.02	3.30	2.99	3.70	0.24	0.38	0.03	0.32
Faecal dm	21.3	12.6	23.6	13.9	2.2	0.44	0.001	0.83
Faecal CFU[c]	24	355	55	569	97	0.22	<0.001	0.36
Protein restriction (1 control, 2 restricted)								
Feed intake, g/d	1030	1024	1035	1011	57	0.95	0.80	0.89
Gain, g/d	421	338	326	261	55	0.14	0.21	0.88
Faecal score[b]	2.85	3.19	2.33	2.37	0.13	<0.001	0.15	0.24
Faecal dm	20.6	15.9	22.2	24.5	1.9	0.005	0.47	0.04
Faecal CFU[c]	149	376	37	248	115	0.31	0.07	0.95
Lupin inclusion (1 control, 2 lupin inclusion)								
Feed intake, g/d	1181	1184	1223	952	103	0.36	0.20	0.19
Gain, g/d	243	384	356	160	70	0.44	0.70	0.02
Faecal score[b]	2.92	2.86	2.83	3.06	0.17	0.71	0.53	0.34
Faecal dm	16.9	16.5	19.4	15.4	1.9	0.71	0.25	0.36
Faecal CFU[c]	81	454	173	257	164	0.69	0.09	0.27
Extra vit. E (1 control, 2 extra vit. E)								
Feed intake, g/d	1030	1042	1067	1144	69	0.32	0.52	0.64
Gain, g/d	438	488	463	381	54	0.32	0.70	0.11
Faecal score[b]	2.45	2.45	2.69	2.72	0.18	0.05	0.94	0.91
Faecal, DM	22.9	20.1	20.6	15.7	2.9	0.05	0.03	0.53
Faecal, CFU[c]	168	282	380	843	183	0.004	0.03	0.16

[a] SE = Standard error of the non-inoculated control group.
[b] 1 = cloddy, 2 = compact, 3 = soft but with shape, 4 = soft and liquid, 5 = watery and dark, 6 = watery and yellow.
[c] CFU = Colony Forming Units of Haemolytic bacteria per g faeces.

Table 3
Antibody responses of piglets weaned at seven weeks of age and allocated to different dietary treatments in combination with *E. coli* inoculation (LS means).

Treatment	1				2					*P*-value [b]		
E. coli	−	+	−	+	−	+	−	+	SE [a]	Treatment	*E. coli*	Day
Day after weaning	0	0	8	8	0	0	8	8				
Feed restriction (1 ad libitum, 2 restricted)												
E. coli AO	2520	4344	2289	8221	4841	3193	2645	4648	497	0.93	0.04	0.74
IgG	989	979	1142	988	994	1030	1136	982	202	0.92	0.51	0.54
IgM	148	176	103	154	138	142	150	134	25	0.78	0.27	0.13
Protein restriction (1 control, 2 restricted)												
E. coli AO	4164	6726	4450	10200	3421	3495	3540	7511	1221	0.23	0.08	0.04
IgG	913	1003	928	1147	826	836	1139	1133	188	0.87	0.38	0.01
IgM	352	315	348	297	349	332	290	313	44	0.80	0.46	0.37
Lupin inclusion (1 control, 2 lupin inclusion)												
E. coli AO	4280	4788	7828	8815	4560	5612	6698	8223	2174	0.99	0.35	0.01
IgG	1415	869	1829	1336	905	1318	1405	1858	290	0.97	0.87	<0.001
IgM	336	339	301	314	327	309	322	331	60	0.99	0.96	0.36
Extra vit. E (1 control, 2 Extra vit. E)												
E. coli AO	3094	3633	5258	4458	3043	3717	4981	7038	987	0.45	0.31	<0.001

Blood samples were obtained at day 0 and 8 after inoculation, and analysed for antibodies to *E. coli* (AO in arbitrary units), IgG (mg/dL), and IgM (mg/dL).
[a] SE = Standard error of the non-inoculated control group day 0 after weaning.
[b] No significant interactions ($P>0.05$).

Table 2) were analysed separately for each of the four dietary factors by a mixed model including block, treatment, inoculation and the treatment by inoculation interaction and using the MIXED procedure of SAS (Littell et al., 1996). Repeated measurements were taken into account by including random effects of i) block and sow, ii) block, sow and piglet and iii) block, sow, piglet and inoculation in the model. Effect of diet was systemic. Note that room and *E. coli* inoculation are completely confounded.

The antibody response parameters measured in serum (see Table 3) were analysed in a model as described above except that day and its interactions were included while block was excluded when only one block was present. Repeated measurements were taken into account as described above except that block was excluded from the random effects when only one block was present. The measures of antibodies to *E. coli* were log-transformed prior to the statistical analysis. Least squares means (LS means) were back-transformed by the exponential function, and back-transformed standard errors were calculated as the back-transformed LS mean multiplied with the log-transformed estimate of the standard error.

To investigate possible archetypes of animals, the response profiles over time were clustered using the "partitioning around mediods" algorithm. Details of this method are described by Kaufman and Rousseeuw (1990). Correlations are Pearson correlation coefficients.

3. Results

The effects of the four investigated dietary factors and the *E. coli* challenge on performance and faecal characteristics are presented in Table 2 and the effects on the antibody response parameters are presented in Table 3. Table 3 also includes the effect of time (day 0 *vs.* day 9).

3.1. Feed restriction

Two piglets died from diarrhoea during the trial. They were littermates and both were on *ad libitum* feeding level and challenged with *E. coli*. No data of the two are included in the analysis. As expected, feed restriction decreased the weight gain ($P=0.004$), while faecal characteristics were unaffected. *E. coli* challenge clearly affected the faecal characteristics ($P\leq0.03$), and there was a tendency for an interaction ($P=0.09$) between feed restriction and *E. coli* challenge with regard to ADG, i.e. the *E. coli* challenge may decrease ADG for piglets on *ad libitum* feeding. Feed restriction did not affect serum IgG and IgM while the serum concentration of antibodies to *E. coli* was the highest ($P=0.04$) in *E. coli* challenged piglets.

3.2. Protein restriction

Two piglets died from diarrhoea during the trial. They were littermates and one was on normal and one was on restricted protein, and they were both challenged with *E. coli*. No data of the two are included in the analysis. There was a tendency that protein restriction decreased weight gain ($P=0.14$). Protein restriction decreased faecal score ($P<0.001$) and increased faecal dry matter ($P=0.005$) while faecal CFU of Haemolytic bacteria was unaffected. There was an interaction between protein restriction and *E. coli* challenge with regard to faecal dry matter ($P=0.04$), i.e. the *E. coli* challenge decreased faecal dry matter for piglets on the control protein level. Protein restriction did not affect serum IgG and IgM while there was a tendency that challenge with *E. coli* increased ($P=0.08$) serum concentration of antibodies to *E. coli*. Serum concentration of antibodies to *E. coli* and IgG increased ($P\leq0.04$) from day 0 to day 8 in experiment.

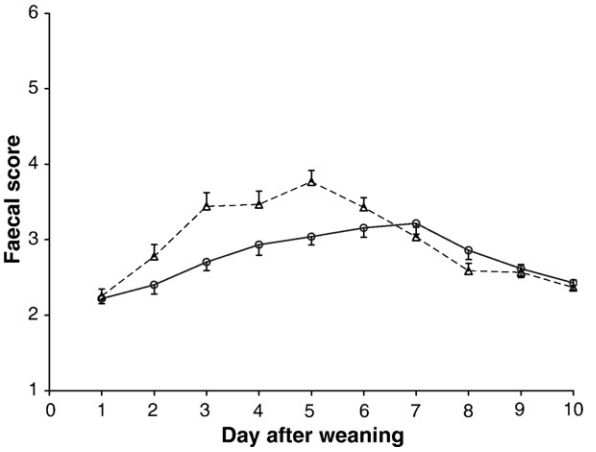

Fig. 1. Faecal score day 1 to 10 after weaning in non-inoculated piglets (circles in solid line) and piglets inoculated with *E. coli* (triangles in broken line) (see text for details). All piglets were included regardless of dietary treatment. SE of each point is indicated.

3.3. Lupin inclusion

There was an interaction between lupin level and *E. coli* challenge with regard to weight gain ($P=0.02$), i.e. the *E. coli* challenge decreased growth in piglets on the diet with lupin. Inclusion of lupin had no effects on feed intake and faecal characteristics. There were no significant effects of lupin inclusion or *E. coli* challenge on the antibody response parameters. Serum concentration of antibodies to *E. coli* and IgG increased ($P\leq0.01$) from day 0 to day 8 in experiment.

3.4. Extra vitamin E

The faecal characteristics were affected by vitamin E, faecal dry matter decreased ($P=0.05$) while faecal score ($P=0.05$) and number of haemolytic bacteria ($P=0.004$) increased in piglets fed supplementary vitamin E. Growth, feed intake and serum concentration of antibodies to *E. coli* were not affected by the extra dietary vitamin E. Serum concentration of antibodies to *E. coli* increased ($P<0.001$) from day 0 to day 8 in experiment. Serum concentrations of IgG and IgM were not included in this experiment.

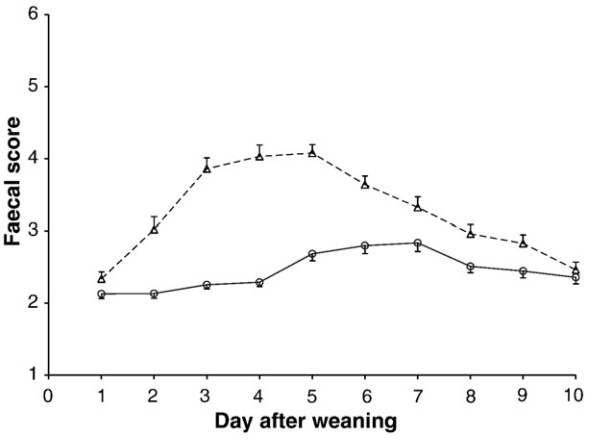

Fig. 2. Faecal score day 1 to 10 after weaning in two clusters of piglets, i.e. piglets developing a diarrhoea-like condition (triangles in broken line) and piglets with only a slight increase in faecal score (circles in solid line) (see text for details). All piglets were included regardless of dietary treatment and inoculation. SE of each point is indicated.

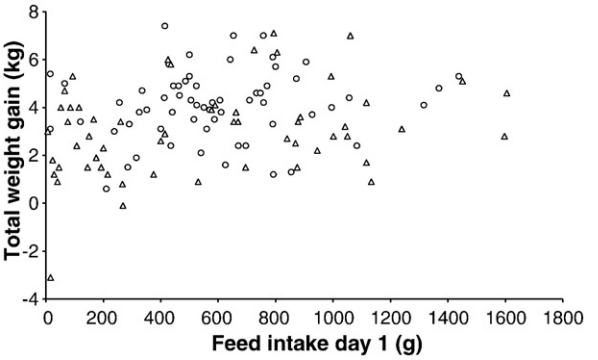

Fig. 3. Total weight gain during the 10-day experimental period as a function of feed intake during the first day after weaning in two clusters of piglets, i.e. piglets developing a diarrhoea-like condition (triangles) and piglets with only a slight increase in faecal score (circles) (see text for details). All piglets were included regardless of dietary treatment and inoculation.

3.5. General effects across experiments

The general effect across all four experiments of the *E. coli* challenge on faecal characteristics was in an adverse direction as exemplified by the faecal score (Fig. 1).

Using the clustering algorithm "partitioning around medoids", the material could be divided into two clusters, one cluster comprising piglets with a diarrhoea-like condition and the other cluster comprising piglets with only a minor increase in faecal score (Fig. 2). The weight gain over the entire experimental period as dependent on feed intake during the first day after weaning is shown in Fig. 3 for the two clusters. The weight gain over the entire experimental period was not affected by feed intake during the first day after weaning. However, the cluster comprising piglets with a diarrhoea-like condition was more frequently represented in the interval below a daily feed intake of 200 g during the first day after weaning than is the cluster comprising piglets with only a minor increase in faecal score indicating that a low feed intake immediately after weaning may predispose for diarrhoea. In line with this indication, the correlation between first day feed intake and average faecal score was −0.66 ($P=0.01$) for the *E. coli* inoculated piglets while it was −0.31 ($P=0.24$) for non-inoculated piglets. Thus, piglets that have a relatively high feed intake immediately after weaning are less predisposed to diarrhoea after an *E. coli* infection.

Creep feed consumption was recorded on a litter basis. Thus, creep feed consumption cannot be associated to measures made on individual piglets but rather to average litter measures. The average daily creep feed consumption per piglet from 28 to 49 days of age was 151 g (SD = 75 g). The correlation between average daily creep feed consumption within a litter and the average faecal score was −0.23 ($P=0.38$) for the non-inoculated piglets and −0.31 ($P=0.24$) for the *E. coli* inoculated piglets. The correlation between average daily creep feed consumption and feed intake during the first day after weaning was 0.48 ($P=0.06$).

4. Discussion

Pigs reared according to organic farming conditions in Denmark are not allowed to be weaned until they are at least 7 weeks of age. Under free-ranging semi-natural conditions, the weaning process would be initiated approximately at this age, but is not fulfilled until 14–17 weeks of age (Jensen, 1986; Petersen, 1994). Thus, although piglets of organic farmed sows are weaned at an older age than are conventionally farmed piglets (approximately 4 weeks in Denmark), milk is still a major nutrient component, and the gastro-intestinal system has to abruptly cope with a diet mixture without milk at the time of weaning.

The challenge with *E. coli* O 149 had variable effects since faecal characteristics were affected to various degrees in the different experiments. It is obvious that the piglets responded variably to the *E. coli* challenge although they were all susceptible to the particular strain of *E. coli*. Thus, although *E. coli* inoculation can be used to introduce a diarrhoea-like condition, the method, as conducted in the present experiment, does not produce uniform experimental subjects. It is possible that methodological modifications with regard to for example time of inoculation relative to weaning, dosage size and frequency may improve the model. Clearly any model that attempts to control the pathogen pressure in individually penned piglets cannot directly represent commercial conditions where oral-faecal exchanges will occur among penmates. Also other housing conditions will likely not cover commercial conditions. Nevertheless, controlled *E. coli* challenge models seem appropriate since it is difficult to study dietary effects on spontaneous weaning diarrhoea that may be have low and variable incidence.

Feed restriction is commonly used as an approach to reduce weaning diarrhoea despite studies that demonstrate intestinal malfunction and damage (McCracken et al., 1999; Spreeuwenberg et al., 2001) as a result of this strategy. On the other hand, restricted feeding day 3 to 8 after weaning has been shown to reduce diarrhoea (Rantzer et al., 1996). Restricted feeding from day 3 will prevent piglets that have not eaten much during the first two stressful days, to engorge on an *ad libitum* available diet when recovering from the stress. Such an engorgement may be associated with digestive upset. In the present experiment, feed restriction had no advantageous effects on faecal score or faecal dry matter content compared to *ad libitum* feeding. Thus, the health benefits necessary to compensate for the inevitable reduction in growth at restricted feeding were absent, and our results therefore do not support the hypothesis that feed restriction after weaning may limit weaning diarrhoea. However, the fact that two inoculated piglets on *ad libitum* feeding died indicates that the results have to be interpreted with caution. In the present experiment, feed restriction was imposed at the individual piglet level. However under commercial conditions with piglets kept in groups, restricted feeding will lead to considerable variation in individual feed intake due to differences in for example speed of feed intake and hierarchy order.

It is well known that the presence of food in the gut is necessary for maintenance of intestinal mucosa (Kelly et al., 1991). It is likewise well known that dietary restriction will lead to villus atrophy (e.g. Pluske et al., 1996). Thus, the risk of intestinal damage and malfunction may be reduced if feed intake after weaning remains uninterrupted. Furthermore, if feed intake of a piglet remains uninterrupted immediately after weaning, i.e., during the first day, under circumstances with maximum stress (transport, foreign location, absence of the sow etc.), it seems unlikely that this piglet will decrease feed intake as a consequence of weaning during the following days. Also the risk of engorgement on an *ad libitum* available diet and the associated risk of digestive upset may be minimal if the piglet has a relatively high feed intake from the day of weaning. Thus, a potential effect of low feed intake on gut health and performance may be associated with a low feed intake during the first day after weaning. Therefore feed intake during the first day after weaning and its effect on the growth performance over the entire experimental period was studied in two clusters of piglets, i.e., one cluster with piglets developing a diarrhoea-like condition and another cluster with piglets characterised by only a minor increase in faecal score. Contrary to our expectation, the overall performance during the experimental period was not significantly affected by feed intake during the first day after weaning. On the other hand, in the analysis including piglets from all four experiments, the cluster comprising piglets with a diarrhoea-like condition is more frequently represented among piglets with a feed intake below 200 g during the first day after weaning than is the cluster comprising piglets with only a minor increase in faecal score. In addition, in *E. coli* infected piglets there was a negative correlation between feed intake during the first day after weaning and faecal score indicating that a relatively high feed intake immediately after weaning will make the piglets less susceptible for diarrhoea. This indicates that although the overall effect of feed intake during the first day after weaning on growth was limited, piglets with a relatively low feed intake immediately after weaning may be more susceptible to diarrhoea. In accordance, Carstensen et al. (2005) found the highest incidence of faecal *E. coli* in the piglets eating the least after weaning at 4 weeks of age.

Protein restriction decreased the degree of diarrhoea and there was a tendency towards a decrease in growth. This result is in accordance with the findings of Eggum et al. (1987) who found a significant decrease in growth. These results thus confirm that a strategy with decreasing the diet protein level immediately after weaning may decrease the degree of weaning diarrhoea. Whether the decrease in the degree of diarrhoea is sufficient to compensate for the decrease in growth performance is debatable.

In organic farming there is some interest in self supply of animal feed. In Denmark, lupin may be a potential source of protein. In addition, lupin is rich in galactose, a substrate for galactane, which has been shown to reduce the number of intestinal *E. coli* (Mathew et al., 1993). Thus potentially lupin might possess the capability to reduce intestinal *E. coli*. This was however not confirmed in this experiment since the number of colonies of haemolytic bacteria was unaffected by inclusion of lupin in the diet. Lupin seems an acceptable source of protein since piglets given 15% lupin in the diet showed performance results comparable to piglets given the control diet.

Vitamin E supplementation is known to stimulate the immune system (Jensen et al., 1988). In line with this, vitamin E deficiency has been found to predispose pigs to *E. coli* infection (Ellis and Vorhies, 1976) while increasing the

vitamin E content in diets for lactating sows has been found to reduce frequency of treatment for weaning diarrhoea and the serum concentration of antibodies to *E. coli* of piglets (Lauridsen and Jensen, 2005). Thus, it was expected that extra vitamin E would have protected against diarrhoea in *E. coli* infected piglets. However, faecal dry matter was lowest and faecal CFU of Haemolytic bacteria was highest among infected piglets given extra vitamin E.

Generally, the antibody response measures in serum increased from the day of weaning to eight days after weaning although the effect was not consistent among the four feeding experiments. The age dependent increase in serum concentration of antibodies to *E. coli* may be attributed to an increase in the invasion of *E. coli* as suggested by Lauridsen and Jensen (2005).

In conclusion, the studied dietary factors could not alleviate a diarrhoea-like condition and at the same time maintain the growth rate. Furthermore, the results indicate that performance after weaning at 7 weeks of age can be improved if piglets achieve a daily feed intake of at least 200 g from the day of weaning.

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