Effects of different quality fishmeal and other protein sources on growth performance of weaned piglets

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Abstract

The effects of 2 different quality fishmeal and 4 other protein sources were evaluated on growth performance of weaned piglets. A total of 288 healthy piglets weaned at 26 d (Yorkshire×Landrace×Duroc, initial body weight 7.7±0.2 Kg) were randomly divided into 6 treatment groups, each group had 6 replicates (8 piglets with same sex ratio per replicate). The control group (SM) was fed a maize–soybean meal based diet without any fishmeal. The FSM diet replaced part of the soybean meal in the control diet with 60g/kg fermented soybean meal. The AFM and SFM diet replaced part of the soybean meal in the control diet with 50g/kg average quality fishmeal or super quality fishmeal respectively. The LM and FLM diet replaced part of the soybean meal in the control diet with 100g/kg linseed meal or fermented linseed meal respectively. All diets are nearly equal on digestible energy (DE), contents of phosphorus and calcium, and contents of nitrogen and the limited amino acids, including lysine, total sulfur amino acids, threonine, tryptophan and arginine. The growth performance, fecal microbial enumeration and serum biochemical parameters were determined. The results showed that, the super fishmeal (SFM) with lower TVB-N value and histamine content is the best protein source from growth performance, and it is better in value than average quality fishmeal (AFM) in all growth parameters. Fermentation could improve the protein quality to a great extent. The fermented soybean meal (FSM) could be used to replace the average quality fishmeal (AFM) but still lower than SFM, while the fermented linseed meal(FLM) even better than soybean meal(SM) except its palatability.

Introduction

Because of the not fully development of the digestive system, weaned piglets is very sensitive to protein sources in their diets. The difference in protein structure and content of the anti-nutritive compounds in protein sources often not only means difference in palatability and digestibility of amino acids in diets, but also could cause greater stress and poorer growth performance during weaning periods.

Normally, fishmeal (FM) is considered to be a high-protein and low anti-nutritive compounds feed ingredient in piglet diets. However, the palatability and digestibility of fish meal may vary significantly, because of the sources and the freshness of the raw material used to produce the fish meal, and processing parameters (i.e. temperature) used (Aksnes and Mundheim, 1997). The quality of fish meal has been shown to greatly affect growth performances and feed efficiency in different species of fish (Pike et al., 1990; Anderson et al., 1993, 1995; Pike, 1993; Moksness et al., 1995; Aksnes and Mundheim, 1997). But little information published about the effects of fishmeal quality on growth performance in weaned piglets.

Recently, some studies have explored the effect of replacing fish meal with cheaper vegetable protein sources such as soybean products in piglet diets on growth performance(Sun et al., 2009). Soybean meal (SBM) is the most commonly used protein source in pig diets. However, a variety of anti-nutritional factors (ANFs) limited the use of soybean meal in weaned piglets (Li et al., 1990; Jiang et al., 2000). For example, trypsin inhibitors can cause growth depression, trypsin inactivation in the gut and adversely affect protein digestion in animals (Holm et al., 1992; Schultze et al., 1993; Hong et al., 2004), glycinin protein and β-conglycinin may elicit an hypersensitivity response in animals were seen as the main reason for the reduction of nutritional value in soybeans (Stokes et al., 1984; Miller et al.,1986; Li et al., 1990). The lower trypsin inhibitor content and higher nutritional value of soybean meal after fermentation with Aspergillus oryzae or other bacterium were reported (Hong et al., 2004; Feng et al., 2007). Kim et al.(2005) showed that soybean meal with A. oryzae could improve growth performance and feed gain ratio (FGR) in pigs. Linseed meal is an oil by-product and contains mucilage, a gel forming carbohydrate fraction present at levels up to 80 g/kg (Fedeniuk and Biliaderis,1994). Mucilage has a high water binding capacity and increases viscosity, in one hand which may result in formation of a biofilm on the intestinal epithelium, providing additional protection of the intestinal mucosa; in another hand which may act as insoluble nonstarch polysaccrides(NSP) and decrease the digestibility of nutrients in diets.

The purpose of this experiment was to compare the effects of 2 grades fishmeal and 4 vegetable protein sources on the growth performance of piglet diets. The protein sources included fishmeal, supper fishmeal, soybean meal, fermented soybean meal, linseed meal and fermented linseed meal.

1. Materials and methods

1.1 protein sources

2.1.1 Average fishmeal（AFM） and Super fishmeal （SFM）was provided by IFFO. Nutrient contents are listed in table 1. And fatty acids profile is listed in table 2. According to the analysis data, the nutrient contents difference between these two fishmeal samples is little. The main difference exist on the total volatile basic nitrogen(TVB-N) and histamine contents, which are closely related to the freshness of fishmeal. The Average fishmeal（AFM） has higher TVB-N value and histamine contents than the Super fishmeal （SFM).

2.1.2 Soybean meal(SM) was bought from commercial oil manufacture. Nutrient contents are listed in table 1.

2.1.3 Fermented Soybean meal (FSM) was prepared on the basis of the above soybean meal. The strain of E.faecalis ANEF01 and Saccharomyces cerevisiae ANSC14 was screened and stored by State Key Lab for Animal Nutrition, China Agricultural University. They had been used for commercial fermentation of feedstuffs for several years. The number of E.faecalis ANEF01 and Saccharomyces cerevisiae ANSC14 in combined culture powder are 1.2×10^8 and 2.6×10^8 respectively. Firstly, 100g of the combined culture powder was mixed with 15L of 30℃ water and 200g sucrose in steel bucket and then cultured at 30℃ for 1hr. The fermentation of soybean meal was taken by soaking the soybean meal with water in a ratio 100:25 (100 part CSM to 25 part water) and then inoculated with 15% the above combined liquid culture (vol/wt), mixed and fermented at 30℃ for 48hr in a plastic container (50 cm x 50 cm x 80 cm) with single-way automatic exhaust valve for exhaustion the biogas but blotting air into the container. After fermentation, the feedstuff samples were dried at 50℃ in an oven for about 1d to about 90% dry matter. The dried samples were subsequently milled fitted with 1-mm mesh screen and stored at room temperature.

2.1.4 Linseed meal(LM) was bought from commercial oil manufacture. Nutrient contents are listed in table 1.

2.1.5 Fermented Linseed meal(FLM) was prepared on the basis of the above soybean meal. The fermentation process and parameters of Linseed meal(FLM)are same to the fermentation of soybean meal(SM).

Table 1 Nutrient content of 6 protein sources (%)

	AFM	SFM	SM	FSM	LM	FLM
Dry Matter (DM)	90.17	90.92	88.72	88.54	88.45	88.45
DE, Mcal/kg	3.95	3.95	3.62	3.97	3.21	3.21
ME, Mcal/kg	3.51	3.51	3.30	3.60	3.01	3.01
NE, Mcal/kg	2.35	2.35	2.09	2.28	2.31	2.31
Total volatile basic nitrogen	129.00	96.90				
Histamine（mg/100g）	384.00	134.00				
Crude protein（CP，N×6.25）	67.40	68.97	43.50	49.01	31.80	31.94
Ether extract (EE)	6.26	8.92	2.60	2.91	0.89	0.92
Crude ash(CA)	15.78	15.34	5.91	6.60	5.63	5.74
Calcium(Ca)	3.39	3.39	0.31	0.34	0.43	0.44
Phosphorus(P)	2.43	2.46	0.65	0.69	0.78	0.78
Asp	6.13	6.03	4.68	4.97	2.68	2.54
Ser	2.86	2.82	2.32	2.45	1.48	1.54
Glu	7.81	8.17	7.65	8.02	5.08	5.64
Thr	3.00	3.07	1.68	1.90	1.02	1.01
Gly	3.47	3.27	1.69	1.79	1.50	1.65
Arg	4.41	4.11	3.81	3.99	2.65	2.97
Ala	4.23	4.21	1.95	2.12	1.59	1.76
Tyr	2.45	1.67	1.74	1.45	0.98	1.39
Pro	3.05	3.25	2.66	2.77	1.46	1.59
Val	3.43	3.48	1.85	1.88	1.22	1.38
Phe	2.17	2.28	2.02	2.02	1.22	1.19
Ile	2.09	2.47	1.74	1.74	1.00	1.10
leu	4.21	4.76	3.30	3.35	1.76	1.85
His	1.46	1.82	1.08	0.93	0.61	0.68
Lys	5.45	5.58	2.64	2.97	1.21	1.29
Met	1.78	1.83	0.58	0.65	0.46	0.47
Cys	0.59	0.62	0.64	0.73	0.41	0.39

Note：The DE, ME and NE contents are calculated values. And all other nutrient contents are determined values.

Table 2 Fatty acids profile of 2 fishmeal sources (mg/g)

Fatty acids	AFM	SFM
C6:0	0.04	0.07
C8:0	0.01	0.01
C10:0	0.01	0.01
C12:0	0.06	0.07
C13:0	0.02	0.02
C14:0	4.37	3.99
C14:1	0.03	0.03
C15:0	0.31	0.34
C16:0	13.41	14.35
C16:1	5.54	3.85
C17:0	0.53	0.66
C18:0	3.32	3.82
C18:1n9c	4.18	4.91
C18:2n6c	0.76	0.85
C18:3n6	0.24	0.23
C18:3n3	0.32	0.52
C20:0	0.15	0.01
C20:1	0.49	0.55
C21:0	0.14	0.18
C20:2	0.05	0.05
C20:3n6	0.16	0.12
C20:4n6	1.00	1.08
C20:3n3	0.03	0.07
C20:5n3	11.61	10.10
C22:0	0.14	0.14
C22:1n9	0.10	0.12
C22:2	0.25	0.06
C23:0	0.43	0.44
C24:0	2.09	1.99
C22:6n3	12.52	16.46
C24:1	0.61	0.88
Total fatty acids	62.92	65.98
unsaturated fatty acid, USFA	37.89	39.88

monounsaturated fatty acid, MUFA	10.95	10.34
polyunsaturated fatty acid, PUFA	26.94	29.54
saturated fatty acid, SFA	25.03	26.10
n-3 polyunsaturated fatty acids	24.48	27.15
n-6 polyunsaturated fatty acids	2.16	2.28
n-3/n-6 ratio	11.33	11.91

1.2 Animal and diet

This study was approved by the Animal Care and Use Committee of China Agricultural University.

A total of 288 healthy piglets weaned at 26 d (Yorkshire×Landrace×Duroc, body weight 7.7±0.2 Kg) were randomly divided into 6 treatment groups, each group had 6 replicates (8 piglets with same sex ratio per replicate). The control group (SM) was fed a maize–soybean meal based diet without any fishmeal. The FSM diet replaced the soybean meal in the control diet with 60g/kg fermented soybean meal. The AFM and SFM diet replaced the soybean meal in the control diet with 50g/kg average quality fishmeal or super quality fishmeal respectively. The LM and FLM diet replaced the soybean meal in the control diet with 100g/kg linseed meal or fermented linseed meal respectively. All diets are nearly equal on digestible energy (DE), contents of phosphorus and calcium, and contents of nitrogen and the limited amino acids, including lysine, total sulfur amino acids, threonine, tryptophan and arginine. All nutrients met or exceeded the recommendations of the Nutrient Requirements of swine (Ministry of Agriculture of the People's Republic of China, NY/T 65-2004). The composition and nutrient levels of the experimental diets were listed in table 3.

Table 3 Composition and nutrient levels of the experimental diets

ingredients	SM	FSM	AFM	SFM	LM	FLM
Corn	56.35	57.25	59.81	59.81	52.70	52.70
Extruded soybean	10.00	10.00	10.00	10.00	10.00	10.00
Milk whey	6.00	6.00	6.00	6.00	6.00	6.00
Soybean meal	23.90	17.00	15.80	15.80	17.40	17.40
Fermented soybean meal		6.00				
Average quality fishmeal			5.00			
Super quality fishmeal				5.00		
Linseed meal					10.00	
Fermented Linseed meal						10.00
Calcium carbonate	1.00	1.00	1.00	1.00	1.00	1.00
Calcium hydrogen phosphate	1.30	1.30	1.00	1.00	1.30	1.30
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30
L-Lysine sulfate[65%]	0.24	0.24	0.20	0.20	0.36	0.36

DL-methionine		0.03	0.03	0.02	0.02	0.04	0.04
Theronine		0.04	0.04	0.03	0.03	0.06	0.06
Vitamin & mineral premix*		0.44	0.44	0.44	0.44	0.44	0.44
Antioxidant		0.01	0.01	0.01	0.01	0.01	0.01
Anti-fungi agent		0.02	0.02	0.02	0.02	0.02	0.02
Zinc oxide		0.30	0.30	0.30	0.30	0.30	0.30
10% colistin		0.05	0.05	0.05	0.05	0.05	0.05
4% enduracidin		0.02	0.02	0.02	0.02	0.02	0.02
Nutrient levels							
DE,#	MC/Kg	3.44	3.46	3.46	3.46	3.40	3.40
ME,#	MC/Kg	3.27	3.29	3.29	3.29	3.23	3.23
NE,#	MC/Kg	2.42	2.44	2.46	2.46	2.42	2.42
Crude protein	%	19.05	19.14	19.05	19.00	19.04	19.07
Lysine,	%	1.19	1.18	1.24	1.24	1.20	1.21
Methionine,	%	0.35	0.35	0.37	0.37	0.38	0.38
Methionine+cystine	%	0.73	0.72	0.72	0.72	0.77	0.77
Tryptophan	%	0.22	0.22	0.22	0.22	0.22	0.22
Theronine	%	0.74	0.73	0.73	0.73	0.74	0.75
Calcium,	%	0.84	0.84	0.92	0.92	0.86	0.86
Phosphorus	%	0.62	0.62	0.65	0.65	0.64	0.64
Non phytic Phosphorus	%	0.40	0.40	0.44	0.45	0.42	0.42

* Vitamin & mineral premix provided were as follows in per kilogram of diet: vitamin A8000 IU, vitamin D3 1200 IU, vitamin E 25 IU, vitamin K3 3.0 mg, vitamin B1 2.0 mg, vitamin B2 4.0 mg, vitamin B6 4 mg, vitamin B12 0.02 mg, niacin 24 mg, calcium pantothenate 10.2 mg, folic acid 0.6 mg/kg, biotin 200 ug, choline chloride 0.5 g, manganese 65 mg, iron 150 mg, zinc 120 mg, copper 220 mg, iodine 0.5 mg and selenium 0.3 mg.

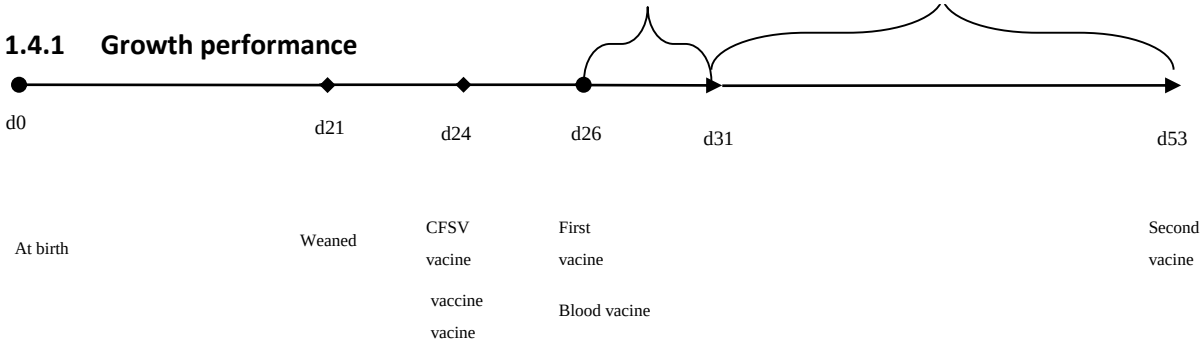
#The DE, ME and NE contents are calculated values. And all other nutrient contents are determined values.

1.3 Animal management

The feeding experiment was conducted in the base of production, education & Research, which was managed by China Agricultural University and Fengze pig farm and breeding company in fuqing city, Fujian province. The pigs were housed in one full closed pig house. During the experimental period, pigs allowed ad libitum access to experimental diets and water. The wet curtain cooling fans were used to keep the air in circulation and the room temperature was kept at 26 ± 2°C. The diets were fed in mash form. The experiment period lasts for 28 days from 26d to 53d after birth, including 5 days pre-trial period. All piglets were taken vaccine at 24d with one dose of Classical Swine Fever Cell Vaccine (Certificated No.（2012）190591084, Winsun pharm Ltd., Guangdong province, China).

1.4 Measurements

1.4.1 Growth performance



Live weight of the pig was recorded at the beginning and at the end of the experiment. Feed consumption was recorded every week. The average daily gain (ADG), average daily feed intake (ADFI) and Feed Conversion Ratio (FCR) were calculated for the whole period.

1.4.2 Fecal consistency and diarrhea incidence

The occurrence of diarrhea for each piglet was visually assessed each morning from 8:00 to 10:00 according to the method of Wu et al. (2012). Scores were 0 = normal, firm feces; 1= possible slight diarrhea; 2 = definitely unformed, moderately fluid feces; or 3 = very watery and frothy diarrhea. A cumulative diarrhea score per diet and day was then calculated (Montagne et al., 2004). The occurrence of diarrhea was defined as maintaining fecal scores of 2 or 3 for two consecutive days. Diarrhea incidence was calculated according to the formula where diarrhea incidence (%) = number of piglets with diarrhea within a treatment/(number of piglets×total experimental days)

×100, where “number of piglets with diarrhea” was the total number of piglets with diarrhea observed each day(Huang et al, 2004).

1.4.3 Feces samples collection and Microbial enumeration

Freshly voided fecal samples were collected in the morning at the last day of feeding trial from 3 piglets of each replicates and samples from same replicates were mixed well. Then all samples were immediately immersed in liquid nitrogen and preserved at−80 ◦C for later microbial analysis. In vitro survival of Lactobacillus, E. coli, total aerobes were determined according to the methods of Mikkelsen et al. (2003) and Guo et al. (2006) with certain modifications. In brief, before enumeration, frozen feces samples were incubated at 4 ◦C for 10 h. Thereafter, 0.5 g of feces was taken from each sample and serially diluted with 1 ml sterile physiological saline, resulting in dilutions ranging from 10^{-1} to 10^{-6} for enumeration. E. coli and total aerobes were cultured on MacConkey agar (Beijing Haidian Microbiological Culture Factory, Beijing, China). Lactobacillus was determined using De Man, Rogosa, Sharpe agar (Oxoid Ltd., Hampshire, UK). Plates of Lactobacillus were incubated inside an anaerobic jar with Anaero Pack Anaero (Mitsubishi Gas Chemical Company, Beijing, China). All plates were incubated at 37 ◦C for 24 h. The microbial enumerations were expressed as log10 colony-forming units per gram.

1.4.4 Antibody and immunoglobin analysis

One specific pig from each replicate selected and labeled by electronic ear tag to collect blood samples via precaval vein on 26d and 53d. Blood samples were kept on ice for approximately 1 h immediately after collection. These samples were then centrifuged at 8000 g for 15 min to separate serum and serum samples were stored at -20 ◦C for biochemical assays.

The indirect hemagglutination (IHA) test was used to detect serum CSFV-specific antibody titer of pigs at 26d and 53d. A commercial IHA kit (Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Science, Lanzhou, China) was used for the test, by following the instruction of the manufacturer. In brief, each serum sample was diluted into a series of solutions (50 uL/well) which contained serum sample at ratio of 1/2, 1/4, 1/8, 1/16, 1/32 and 1/64. Similarly, CSFV antibody-negative serum was diluted into a series of solutions (1/2, 1/4, 1/8 and 1/16, 50 mL/well), while CSFV antibody-positive serum was diluted at 1/2, 1/4, … 1/512(50 uL/well). All wells were then added 25 uL of antigen-coated erythrocytes and incubated at 25 ◦C for 1h. Afterwards, the wells were categorized as follows: ‘‘-’’ no agglutination, 100% sediment of erythrocytes on the bottom of wells; ‘‘+’’, 25% hemaggluti nation; ‘‘++’’, 50% hemagglutination; ‘‘+++’’, 75% hemagglutination; ‘‘+++’’, 90%-100% hemagglutination. It was qualified that no erythrocytes agglutination (‘‘-’’) or 25% hemagglutination (‘‘+’’) at wells with CSFV antibody-negative serum, while all wells with CSFV antibody-positive serum appeared as 50% hemagglutination (‘‘++’’) or 75% hemagglutination (‘‘+++’’). In comparison with CSFV antibody-positive serum wells, when test serum sample appeared 50% hemagglutination (‘‘++’’), its maximum dilution ratio was used to calculate the antibody titer, which was expressed as Geometric Mean Titer (Log 2).

In addition, an immune transmission turbidity assay was performed to detect serum immune globulins of pigs at 53d following the recommended protocol from the manufacturer (ELIKAN Bio-tech Inc., Zhejiang, China). The assay includes sets of three U-bottom plate wells for blank, standards and samples. Each serum sample was analyzed in duplicates. The blank, standard and sample wells each were added 3 mL of deionized water, standards and serum samples, respectively, and then 300 mL of complete reagent which contained 100 mmol/L Tris buffer, 40g/L polyoxyethylene, 0.95g/L preservative and goat-anti-human IgG, IgA, IgM antibody. Afterwards, the assay was incubated at 37 ◦C for 10 min. The optical density (OD) values of standards and serum samples at 600 nm (IgG) or 340 nm (IgA, IgM) were measured (MuLtisKan MK3, Thermo Labsystems, USA). Serum immune globulins concentration (g/L) are calculated as standard concentration (g/L) multiplied by OD value of serum sample and then divided by OD value of standard. The concentrations of total protein (TP), albumin (ALB) and globulin (GLB) in the serum were measured with a commercial assay kit (Nanjing Jiancheng Biological Product Co., Ltd., China). All of the analyses were conducted according to the manufacturer’s instructions.

1.4.5 Profit efficiency analysis

Profit efficiency analysis were done on the basis of the current price of feedstuff and piglets. The feed cost for each unit of weight gain was calculated.

1.5 Data statistical analyses

All data were analyzed using the one-way analysis of variance (ANOVA) using the SPSS 12.0 software with Duncan's method for difference comparation. Effects were considered to be significant when $P < 0.05$. The results were expressed as mean ± SD.

2. Results

2.1 Growth performance

There is no significant difference on initial body weight (IBW) among treatments, but there are extremely significant differences on final body weight (FBW) (Table 4). Pigs fed SFM had the highest FBW and it is significant higher than those fed FLM, SM and LM (P<0.05), but no significant difference exist among SFM, AFM and FSM (P>0.05). Pigs fed FLM had nearly similar FBW with those of SM, and it is significant higher than those of LM.

There is extremely significant difference on ADFI (P<0.01) and ADG (P<0.01), significant difference on FCR (P<0.05) among six different protein sources (Table 4). Pigs fed SFM had the highest ADFI but no significant difference exist between SFM and other treatments (P>0.05) except LM(P<0.05), while pigs fed LM had the lowest ADFI and it is lower than all of those fed other protein sources (P<0.05). Pigs fed SFM had the highest ADG and it is significant higher than those fed SM, FLM and LM (P<0.05), but no significant difference exist among SFM, AFM and FSM (P>0.05). Pigs fed LM had the lowest ADG than those fed all of other protein sources (P<0.05). Pigs fed SFM had the best FCR (F/G) and it is significant higher than those fed SM and LM (P<0.05), but not significant better than those fed AFM, FSM and FLM (P>0.05). Pigs fed SM had the worst FCR (F/G) and it was worse than those fed other protein sources (P<0.05) except SFM (P>0.05).

In conclusion, the super quality fishmeal (SFM) is the best protein from growth performance, and it is better in value than average quality fishmeal (AFM) in all growth parameters. Fermentation could improve the protein quality to a great extent. The fermented soybean meal (FSM) could be used to replace the average quality fishmeal (AFM), while the fermented linseed meal(FLM) even better than soybean meal(SM) except its palatability.

Table 4: Effect of dietary protein sources on growth performance of weaned piglets* (n=6)

Item	SM	FSM	AFM	SFM	LM	FLM	P value
IBW （kg）	7.71±0.06	7.71±0.04	7.71±0.03	7.73±0.04	7.73±0.02	7.72±0.02	0.998
FBW (kg)	17.36±0.25b	17.59±0.28bc	17.77±0.31bc	18.31±0.29c	16.25±0.18a	17.44±0.18b	0.000
ADFI(g/d)	628.0±14.8b	620.0±15.2b	627.2±15.6b	637.5±10.2b	548.0±10.5a	600.0±13.8b	0.001
ADG (g/d) #	346.2±8.5b	354.0±9.8bc	359.2±11.5bc	377.3±10.3c	304.3±6.0a	347.0±7.1b	0.000
FCR(F/G)	1.81±0.02b	1.76±0.02ab	1.75±0.02ab	1.69±0.04a	1.80±0.02b	1.73±0.03ab	0.047

**Values are listed as means ± standard error. Within arrow, values with different super script letters mean significant differences (P< 0.05).*

#IBW ：Initial Body Weight ；FBW: Final Body Weight; ADFI: Average Daily Feed Intake ；ADG: Average Daily Gain ；FCR: Feed Conversion Ratio(F/G); SM: soybean meal diet; FSM: fermented soybean meal diet; AFM: average quality fishmeal diet; SFM: super quality fishmeal diet; LM ：linseed meal diet; FLM fermented linseed meal diet.

2.2 Fecal consistency and diarrhea incidence

There is no significant difference on diarrhea incidence and diarrhea score (P>0.05) among all of protein sources (Table 5).

Table 5: Effect of dietary protein sources on diarrhea incidence of weaned piglets* (n=6)

item	SM#	FSM	AFM	SFM	LM	FLM	P value
Diarrhea incidence (%)	1.97±0.91	2.55±1.49	2.31±1.05	3.13±1.06	3.35±2.28	2.31±1.15	0.98
Diarrhea score	0.75±0.05	0.73±0.01	0.60±0.06	0.55±0.09	0.61±0.08	0.61±0.05	0.34

**Values are listed as means ± standard error. Within arrow, values with different super script letters mean significant differences (P< 0.05).*

 # SM: soybean meal diet; FSM: fermented soybean meal diet; AFM: average quality fishmeal diet; SFM: super quality fishmeal diet; LM: linseed meal diet; FLM: fermented linseed meal diet.

2.3 Microbial enumeration in feces

There is no significant difference on counts of total aerobes, E.coli, and Lactobacillus (P>0.05), but exist significant difference on the ratio of counts between Lactobacillus and E. coli（L/E） (P<0.05) among six different protein sources (Table 6). Pigs fed LM had the lowest L/E ratio and it is significant lower than those of FSM, FLM and SFM (P<0.05). Fermentation of SM or LM could increase the L/E ratio in the feces samples. FSM, FLM and SFM were the better protein source choices from the balance of intestinal microflora.

Table 6: Effect of dietary protein sources on counts of selected bacteria (lg CFU/g of feces) (n=6)

Item	SM#	FSM	AFM	SFM	LM	FLM	P value
total aerobes	8.49±0.11	8.76±0.08	8.75±0.12	8.62±0.10	8.62±0.21	8.60±0.14	0.716
E. coli	5.64±0.16	5.38±0.08	5.76±0.22	5.50±0.19	6.08±0.08	5.54±0.17	0.058
Lactobacillus	8.76±0.12	9.00±0.01	8.91±0.12	8.89±0.05	8.87±0.11	9.03±0.08	0.352
L/ E	3.12±0.14ab	3.63±0.09b	3.15±0.29ab	3.39±0.20b	2.79±0.16a	3.48±0.09b	0.026

**Values are listed as means ± standard error. Within arrow, values with different super script letters mean significant differences (P< 0.05).*

 # L/E: the ratio of counts between Lactobacillus and E. coli ; SM: soybean meal diet; FSM: fermented soybean meal diet; AFM: average quality fishmeal diet; SFM: super quality fishmeal diet; LM: linseed meal diet; FLM: fermented linseed meal diet.

2.4 Serum biochemical parameters

There is no significant difference on TP, GLB, A/G, IgG, IgA and IgM (P>0.05),but ALB(P<0.05) among six different protein sources (Table 7). Serum ALB in AFM or SFM is significant lower than those of LM and FLM, while it was also lower than those of SM and FSM but no significant difference existed. Fermentation of SM or LM couldn't change its response on serum ALB very much.

Table 7: Effect of dietary protein sources on serum biochemical parameters (n=6)

Item	SM#	FSM	AFM	SFM	LM	FLM	P value
TP(g/L)	59.19±1.53	63.05±2.23	58.62±2.05	59.06±1.06	64.54±2.16	62.89±1.24	0.087
ALB(g/L)	27.75±0.37^{abc}	28.0±0.82^{abc}	26.25±1.01^{a}	26.63±0.82^{ab}	29.75±0.62^{c}	28.75±0.65^{c}	0.019
GLB(g/L)	31.44±1.69	35.05±2.05	32.37±2.10	32.44±1.16	34.79±1.99	34.14±1.10	0.600
A/G	0.9±0.055	0.82±0.046	0.83±0.062	0.83±0.048	0.87±0.053	0.85±0.034	0.84
IgG(g/L)	7.45±0.21	8.34±0.24	8.10±0.38	8.57±0.30	8.11±0.40	8.44±0.32	0.18
IgA(g/L)	1.15±0.08	1.16±0.05	1.10±0.06	1.15±0.06	1.16±0.06	1.12±0.60	0.97
IgM(g/L)	0.96±0.03	0.94±0.045	0.88±0.025	0.90±0.035	0.90±0.026	0.87±0.40	0.8

**Values are listed as means ± standard error. Within arrow, values with different super script letters mean significant differences (P< 0.05).*

 # TP: serum total protein; ALB:serum albumin; GLB: serum globulin; A/G: ratio of ALB/ GLB; SM: soybean meal diet; FSM: fermented soybean meal diet; AFM: average quality fishmeal diet; SFM: super quality fishmeal diet; LM: linseed meal diet; FLM: fermented linseed meal diet.

2.5 Specific immune response

Table 8: Effect of dietary protein sources on CSFV specific antibody in weaned piglets (n=6)

Item	SM#	FSM	AFM	SFM	LM	FLM	P value
Antibody tilter at 26d	2.38±0.19	2.46±0.38	2.23±0.07	1.91±0.16	1.98±0.09	1.82±0.18	0.155
Antibody tilter at 53d	1.12±0.13	1.20±0.05	1.26±0.07	1.01±0.12	1.02±0.07	1.09±0.08	0.418
Diff. between 26d and 53d	1.26±0.15	1.25±0.34	0.98±0.11	0.90±0.08	0.96±0.06	0.73±0.20	0.248

Values are listed as means ± standard error. Within arrow, values with different super script letters mean significant differences (P< 0.05).

 # SM: soybean meal diet; FSM: fermented soybean meal diet; AFM: average quality fishmeal diet; SFM: super quality fishmeal diet; LM: linseed meal diet; FLM: fermented linseed meal diet.

There is no significant difference on antibody tilter at 26d, 53d and the difference between them (P>0.05). (Table 8). However, the antibody tilter at 26d is more dependent on the maternal antibody. After the first taken of CSFV vaccine, normally the antibody tilter will firstly decreases and then increases. If we want to know the specific immune response of CSFV, we need to carry out a serial determination at later time.

2.6 Profit efficiency analysis

Although both of the fishmeal diets had higher feed formulation cost and feed cost for unit of weight gain, the whole growth Marathon is just past one third and the potential programming effects may be aroused during the grower and finisher period.

Table 9: Effect of dietary protein sources on feed cost for unit of weight gain

Item	SM	FSM	AFM	SFM	LM	FLM
Average FCR(F/G)	1.81	1.76	1.75	1.69	1.8	1.73
Feed formulation cost(RMB ￥/kg)	3300	3357	3544	3644	3243	3313
Formulation cost(US $/kg)	532	541	572	588	523	534
feed cost for unit of weight gain(US$/kg)	0.96	0.95	1.00	0.99	0.94	0.92

Values are listed as means ± standard error. Within arrow, values with different super script letters mean significant differences (P< 0.05).

 # SM: soybean meal diet; FSM: fermented soybean meal diet; AFM: average quality fishmeal diet; SFM: super quality fishmeal diet; LM: linseed meal diet; FLM: fermented linseed meal diet. The arrival price of SM, FSM, AFM, SFM, LM and FLM is 4000 RMB ￥/ton, 5200 RMB ￥/ton, 10000 RMB ￥/ton, 12000 RMB ￥/ton, 2800 RMB ￥/ton, 3500 RMB ￥/ton respectively. The exchange rate of RMB ￥ against the U.S.$ is 6.2.

3. Conclusion

Conclusively, the super fishmeal (SFM) with lower TVB-N value and histamine content is the best protein source from growth performance, and it is better in value than average quality fishmeal (AFM) in all growth parameters. Fermentation could improve the protein quality to a great extent. The fermented soybean meal (FSM) could be used to replace the average quality fishmeal (AFM) but still lower than SFM, while the fermented linseed meal(FLM) even better than soybean meal(SM) except its palatability.

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