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Short Communication

Influence of fermented fish meal supplementation on growth performance, blood metabolites, and fecal microflora of weaning pigs

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ABSTRACT - This study was conducted to estimate the effect of dietary supplementation with fermented fish meal on growth performance, blood metabolites, and fecal microflora in weaning pigs. A total of 180 weaned pigs ((Landrace × Yorkshire) × Duroc; with average body weight of 6.0 kg) were randomly distributed among three dietary treatments (0, 0.2, and 0.5% fermented fish meal) in three replicate pens (20 heads per pen) in a completely randomized trial over three weeks. Addition of fermented fish meal to weanling pig diets had a linear effect on average feed intake and a quadratic trend on final body weight, average daily gain, and gain:feed ratio throughout the whole period (but not initial body weight). Hematocrit, monocyte, immunoglobulin G, and blood urea nitrogen levels responded linearly and quadratically with increasing levels of dietary fermented fish meal. Moreover, we found a linear correlation between the diets and lymphocyte and insulin levels among the different dietary treatments. In contrast, red blood cells, white blood cells, hemoglobin, insulin-like growth factor 1, and glucose levels were not affected by diets with different levels of fermented fish. During the experimental period, diets with 0.2% and 0.5% fermented fish meal showed a reduction in *Salmonella enterica* and *Escherichia coli* populations (but not *E. coli* populations at week 3) that were linear, quadratic, or both, compared with controls. In particular, there was a significant reduction in *S. enterica* population when pigs were fed 0.5% fermented fish meal over the period of 3 weeks). Dietary supplementation with 0.2% and 0.5% fermented fish meal can be used as a protein source to improve growth performance and the parameters chosen for the blood profile, which reduces harmful microorganisms in the feces of weanling pigs.

Key Words: growth factor, hormone, immune response, pig

Introduction

Newly weaned pigs are very sensitive to the quality of their feed. The wrong feed can result in a lower feed intake and a reduced growth rate due to the incomplete development of their digestive system (Zhang et al., 2003). In particular, certain sources of dietary proteins can cause these problems by producing an allergic response (Bimbo and Crowther, 1992; Maxwell et al., 2003). At this point in their development, pigs require a good source of protein and amino acids in their diets; and there are increasing concerns about finding the most practical protein source for newly weaned pigs that will have the greatest positive influence on the function and structure of their digestive tract. An animal protein source that is widely used for early-weaned pigs (as early as 19-21 days) is fish meal. Fish meal is a very digestible protein source, with a high mineral content and low fiber, that is commonly used to stimulate feed intake (FAO, 2001; Kim and Easter, 2001; Jones et al., 2015). The amino acid composition of fish meal protein is very similar to both milk of sow and body tissue of piglets (Fowler, 1997). Wang et al. (2009) showed clear improvements in growth performance when weanling piglets were fed diets containing fish meal. Other reports have also suggested that adding fish meal to the diet of farmed animals could provide several advantages to animal health, including improved immunity against disease, higher survival and growth, and reduced incidence of deformities (FAO, 1986).

However, falling supply of and rising demand for fish meal has led to uneconomically high prices. In part, this is because the quality of the fish meal depends on the type and species of fish, its freshness, and the processing of methods. Recent approaches advocating the use of fish

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byproduct or green algae have focused on fermentation to improve the quality of the animal feed for pig production. Thus, we hypothesized that fermented fish meal with microorganisms would improve the growth performance in weanling pigs as the partial replacement of high-quality protein. The objective of our study was to further evaluate the effect of dietary supplementation with fermented fish meal on growth performance, blood metabolites, and fecal microflora in weanling pigs.

Material and Methods

The animal experimental protocols were conducted according to the guidelines of the Animal Care and Use Committee of Gyeongsang National University, Jinju, South Korea. A total of 180 weaned pigs ((Landrace × Yorkshire) × Duroc; mixed sex; weaned at 21±1d of age) with initial body weight of 6.0±0.2 kg were randomly distributed among three dietary treatments of three replicate pens (20 weanling pigs per pen) in a completely randomized trial over three weeks. The dietary treatments comprised corn-soybean meal-based diets with 0% (control), 0.2%, and 0.5% fermented fish meal. The commercial diet was used throughout the experiment period (Table 1).

Fish meal, mainly containing cod (*Gadus macrocephalus*) byproduct, was purchased from Dongchang, Busan, South Korea. To obtain fermented fish meal, fresh fish meal (50%), rice bran (30%), and wheat bran (20%) were mixed and 1% of micro-organisms (*Lactobacillus acidophilus*, *Bacillus subtilis,* and *Saccharomyces cerevisiae*) was applied to meet the application ratio at 1.2×10^5 cfu/g (Table 2). Then, the mixture was fermented at 39 °C for 72 h. The fermented fish meal was then dried by enforced-air fan and ground into powder.

The pigs were weaned and housed in an environmentally controlled and slatted-floor facility in 20 pens (2.0 m × 1.8 m). Each pen had one self-feeder and a nipple waterer to allow for *ad libitum* access to feed and water. The temperature was maintained at about 32 °C during the first week and lowered by 2 °C per week down to 28 °C in the third week post-weaning. The relative humidity was kept at 60~70% during the whole period. Each pig was weighed at the beginning (1 d) and the end (21 d) of the experiment period to calculate average daily gain (ADG). Feed intake was also recorded weekly for each pen to measure average daily feed intake and gain:feed ratio (G:F). To determine the amounts of dry matter (DM), crude protein, ether extract, and crude ash, the experimental diets were analyzed in accordance with the methods described by the AOAC (1995) (Table 1).

After three weeks, two pigs from each pen were bled via jugular venipuncture. Blood samples were collected in 5-mL non-heparinized vacuum tubes and 5-mL vacuum tubes containing K_3EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA) to obtain whole blood and serum, respectively. The counts for red blood cells (RBC), white blood cells (WBC), hematocrit (Hct), hemoglobin (Hgb), lymphocytes, and monocytes in the whole blood samples were determined, using an automatic blood analyzer (ADVIA 120, Bayer, Tarrytown, NY, USA). In addition, the samples were centrifuged at 3,000 × *g* for 15 min at 4 °C to separate the serum. Serum immunoglobulin G (IgG) level was determined using ELISA kits, while the concentrations of insulin-like growth factor 1 (IGF-1) and insulin were determined using radioimmunoassay kits. Serum samples were also used to determine blood glucose and blood urea nitrogen (BUN) levels.

To measure *Salmonella enterica* and *Escherichia coli* loads, fecal samples (100 g) were collected weekly from each pen at four random locations and immediately analyzed.

Table 1 - Ingredients and chemical compositions of basal diets (% of dry matter basis or as stated)

Item	Concentrate
Ingredient	
Ground corn	64.95
Soybean meal	20.00
Wheat bran	2.90
Tallow	3.87
Salt	0.12
Molasses	4.00
Tricalcium phosphorous	0.75
Lycine	0.36
Methionine	0.04
Choline chloride	0.04
Mineral premix[1]	2.00
Vitamin premix[2]	1.00
Total	100.0
Energy value and chemical composition	
Digestible energy (kcal/kg)	3,500
Ca	0.65
P	0.80
Lysine	0.97
Dry matter	89.3
Crude protein	24.5
Ether extract	8.05
Crude ash	6.77

[1] Supplied per kg diet: Fe, 100 mg; Cu, 50 mg; Zn, 25 mg; Mn, 15 mg; Co, 2.5 mg; I, 0.1 mg.
[2] Supplied per kg diet: vitamin A, 25,000 IU; vitamin D3, 5,000 IU; vitamin E, 30 mg; thiamin, 1.0 mg; riboflavin, 15 mg; vitamin B6, 2.5 mg; niacin, 75 mg.

Table 2 - Microbial counts of fermented fish meal

Item	Fermented fish meal
Lactic acid bacteria (log10 cfu/g)	7.15
Bacillus subtilis (log10 cfu/g)	7.30
Yeast (log10 cfu/g)	7.00

Ten grams of samples were weighed and placed into a stomacher bag with 100 mL of phosphate-buffered saline buffer (pH 7.4). Samples were homogenized thoroughly for 1 min. Serial 10-fold dilutions (in 0.1% peptone solution) of the fecal samples were then plated onto Difco TM SS agar (Becton, Dickinson and Company, Sparks, MD, USA) and Difco TM Violet Red Bile agar (Becton, Dickinson and Company, Sparks, MD, USA), respectively. Difco TM SS agar and Difco TM Violet Red Bile agar plates were incubated for 24 h at 37 °C. After being removed from the incubator, the colonies, as average colony-forming units (cfu)/g litter, were counted immediately at week 1, 2, and 3, respectively.

All data were subjected to ANOVA using the GLM procedure of the SAS package program (Statistical Analysis System, version 8). The IML procedure was used to develop linear and quadratic coefficients for data with unequal spacing. Tukey's test was also used to identify treatment means to compare the difference between means. Significance of treatment effects was declared at P<0.05.

Results

Adding fermented fish meal to weanling pig diets had a linear effect (P<0.05) on average feed intake and a quadratic effect (P<0.05) on final body weight (FBW), ADG, and G:F throughout the period. There was no significant difference among diets (linear or quadratic; P>0.05) with the initial body weight (Table 3). Growth performance was greater in the diet with 0.2% fermented fish meal than in the other treatments.

Overall, values on blood metabolites showed differences among all supplemented diets (Table 4). Hematocrit, monocyte, IgG, and BUN responded linearly and quadratically (P<0.05) with increasing levels of dietary fermented fish meal. Moreover, differences (P<0.05) were detected linearly among diets for lymphocytes and insulin. However, RBC, WBC, Hgb, IGF-1, and glucose were not affected by diets with different levels of fermented fish meal over the three-week period (linear and quadratic; P>0.05).

During the experimental period, diets with 0.2% and 0.5% fermented fish meal showed reductions in fecal microbes that were linear, quadratic, or both, compared with controls (Table 5). In particular, there was a significant reduction in *S. enterica* and *E. coli* populations when weanling pigs were fed the 0.5% fermented fish meal at week 1 and week 2. However, no differences (linear and quadratic; P>0.05) among treatments were found in *E. coli* populations at week 3.

Table 3 - Growth performances of weaning pigs fed fermented fish meal

Item	Fermented fish meal[1] (%)			SEM	Contrast	
	0	0.2	0.5		Linear	Quadratic
IBW (kg)	5.99	6.06	5.94	0.257	0.827	0.698
FBW (kg)	11.60b	15.20a	14.40a	0.391	0.190	0.002
ADG (kg)	0.27b	0.44a	0.40a	0.017	0.169	0.001
AFI (kg)	0.36c	0.39b	0.41a	0.006	0.001	0.167
G:F	0.75c	1.13a	0.98b	0.039	0.161	0.001

[1] Substitution of 0, 0.2, and 0.5% of concentrate with fermented fish meal.
IBW - initial body weight; FBW - final body weight; ADG - average daily gain; AFI - average feed intake; G:F - gain to feed ratio; SEM - standard error of the mean.
a-c - Means in the same row with different letters differ significantly (P<0.05).

Table 4 - Blood metabolites of weaning pigs fed fermented fish meal

Item	Fermented fish meal[1] (%)			SEM	Contrast	
	0	0.2	0.5		Linear	Quadratic
RBC (10^6/μL)	4.89	4.83	4.98	0.510	0.936	0.790
WBC (10^3/μL)	12.40	10.90	11.90	0.991	0.255	0.755
Hct (%)	31.80ab	30.40b	35.40a	1.419	0.032	0.030
Hgb (g/dL)	9.32	9.55	9.07	0.214	0.191	0.204
Lymphocyte (%)	52.60ab	64.60a	47.10b	5.323	0.050	0.177
Monocyte (%)	4.27b	5.77a	6.48a	0.389	0.008	0.004
IgG (mg/dL)	251.70ab	230.90b	300.80a	18.32	0.009	0.016
IGF-1 (mg/mL)	156.80	134.50	168.80	27.05	0.342	0.476
Insulin (μU/mL)	0.48b	1.07a	0.65b	0.080	0.001	0.240
Glucose (mg/dL)	120.00	125.80	121.20	7.527	0.688	0.942
BUN (mg/dL)	8.22b	11.90a	11.50a	1.038	0.014	0.022

[1] Substitution of 0, 0.2, and 0.5% of concentrate with fermented fish meal.
RBC - red blood cell; WBC - white blood cell; Hct - hematocrit; Hgb - hemoglobin; IgG - immunoglobulin G; IGF-1 - insulin-like growth factor type-1; BUN - blood urea nitrogen; SEM - standard error of the mean.
a,b - Means in the same row with different letters differ significantly (P<0.05).

Table 5 - Effects of dietary inclusion of fermented fish meal on *Salmonella* and *E.coli* counts of weaning pig

Item	Fermented fish meal[1] (%)			SEM	Contrast	
	0	0.2	0.5		Linear	Quadratic
Week 1						
Salmonella enterica (log10 cfu/g)	6.08a	5.96a	5.12b	0.166	<.0001	0.052
E. coli (log10 cfu/g)	6.58a	6.51a	5.50b	0.089	<.0001	0.001
Week 2						
Salmonella enterica (log10 cfu/g)	5.36a	3.78b	3.89b	0.189	0.001	0.001
E. coli (log10 cfu/g)	6.25a	5.45ab	4.82b	0.385	0.009	0.499
Week 3						
Salmonella enterica (log10 cfu/g)	3.72a	3.58b	3.46b	0.058	0.001	0.391
E. coli (log10 cfu/g)	4.32	3.88	4.31	0.200	0.779	0.061

[1] Substitution of 0, 0.2, and 0.5% of concentrate with fermented fish meal.
SEM - standard error of the mean.
a,b - Means in the same row with different letters differ significantly (P<0.05).

Discussion

Our study finds direct evidence that the inclusion of fermented fish meal improves the growth performance of weanling pigs. Similarly, Kim and Easter (2001) reported that the ADG in young pigs, between 3 and 5 weeks old, was increased by diets with either mackerel or herring fish meal compared with other feed supplements. Improvement in the feed efficiency of weanling pigs due to 5.0% fish byproduct supplementation was also reported by Noh et al. (2014). In our study, the reasons for the improved growth performance of weanling pigs appears to be that fish meal is a protein source with high nutrient digestibility (Jones et al., 2015). In addition, fermented fish meal has been reported to offer benefits for weaning pigs by improving flavor and enriching the feed with desirable metabolites produced by the microorganisms (Buckenhüskes et al., 1990; Cho and Kim, 2011). There were no effects on FBW and ADG between supplementation levels of fermented fish meal. However, pigs fed diet with 0.5% fermented fish meal had greater average feed intake compared with pigs fed 0.2%. These results lead to the greater G:F in 0.2% fermented fish meal compared to 0.5%. This improvement might be due to the nutrient digestibility enhance at 0.2% fermented fish meal. In our study, the parameters we chose for the blood profile (Hct, lymphocyte, monocyte, IgG, insulin, and BUN) had a greater effect on the metabolism and immune status in weanling pigs fed on diets with 0.2% and 0.5% fermented fish meal. In other words, elevated blood metabolites might allow better utilization of fermented fish meal due to improved gut flora in the weanling pig, which might affect lymphocyte, monocyte, and IgG levels. For example, lymphocyte proliferation is an important phase in determining cell immunity and clinical immune function of the animal body (Lafuente et al., 2003). According to Zinnerman (1998), the presence of immunoglobulin had a positive effect on immunity. Likewise, glucose and BUN are important for growth. However, there is little or no research evaluating the effect of fermented fish meal on blood profiles; therefore, more evidence is needed to confirm our results. In another study using fermented soybean meal, Liu et al. (2007) reported decreased serum IgG and lowered whole blood and spleen lymphocyte proliferation in weaned piglets. In the case of fermented fish meal, the blood parameters selected appear to be correlated with growth performance of weanling pigs.It has been well documented that gastrointestinal microflora affects animal production because the activation of the gastrointestinal immune system significantly affects the intestinal morphology and the ability to digest and absorb nutrients in pigs (Yan et al., 2012; Liu, 2015). Consequently, the results in our study indicate that fecal microflora is closely related to the production performance in weanling pigs. Fermented fish meal reduces the populations of *S. enterica* and *E. coli* by creating gut micro-ecological conditions that suppress harmful microorganisms or favor beneficial microorganisms, as previously suggested by Lee et al. (2014). Our results support the findings of Noh et al. (2014), who indicated that diets supplemented with 5.0% citrus pulp, fish byproduct, and *B. subtilis* fermentation biomass have the potential to improve fecal microflora of weanling pigs.

Conclusions

Feeding fermented fish meal in increasing dietary levels (0.2% and 0.5%) to weanling pigs is an effective protein source to improve growth performance and reduce harmful microorganisms (*S. enterica* and *E. coli*) in the feces. In addition, hematocrit, lymphocyte, monocyte, immunoglobulin G, insulin, and blood urea nitrogen have a greater effect on metabolism and immune status in weanling pigs fed diets with 0.2% and 0.5% fermented fish.

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References

AOAC - Association of Official Analytical Chemist. 1995. Official method of analysis. 15th ed. Association of Official Analytical Chemist, Washington, DC.

Bimbo, A. P. and Crowther, J. B. 1992. Fish meal and oil: Current uses. Journal of the American Oil Chemists' Society 69:221-227.

Buckenhüskes, H.; Jensen, H. A.; Andersson, R.; Fernandez, A. G. and Rodrigo, M. 1990. Fermented vegetables. p.162-187. In: Processing and quality of foods in food biotechnology. Zeuthen, P.; Cheftel, J. C.; Eriksson, C. Gormley, T. R.; Linko, P. and Paulus, K., eds. Avenues to Healthy and Nutritious Products. Elsevier, London.

Cho, J. H. and Kim, I. H. 2011. Effects of fermented fish meal on N balance and apparent total tract and ileal amino acid digestibility in weaning pigs. Journal of Animal and Veterinary Advances 10:1455-1459.

FAO - Food and Agriculture Organization of the United Nations. 1986. The production of fish meal and oil. FAO Fisheries Technical Paper 142. Fisheries Technical Division, Food and Agriculture Organization of the United Nations, Rome. 63p.

FAO - Food and Agriculture Organization of the United Nations. 2001. Windsor, M. L. Fishmeal. Department of Trade and Industry, Torry Research Station. Torry advisory note No. 49 (FAO in partnership with Support unit for International Fisheries and Aquatic Research, SIFAR).

Fowler, V. R. 1997. Fishmeal in the diets of pigs. Journal of Animal Science 66 (Suppl. 1):320(abstr.).

Jones, A. M.; Woodworth, J. C.; Goodband, R. D.; Tokach, M. D.; Dritz, S. S. and DeRouchey, J. M. 2015. Effect of fish meal source on nursery pig performance. Kansas Agricultural Experiment Station Research Reports 1:1-13.

Kim, S. W. and Easter, R. A. 2001. Nutritional value of fish meals in the diet for young pigs. Journal of Animal Science 79:1829-1839.

Lafuente, M. J.; Martin, P.; Garcia-Cao, I.; Diaz-Meco, M.T.; Serrano, M. and Moscat, J. 2003. Regulation of mature T lymphocyte proliferation and differentiation by Par-4. The EMBO Journal 22:4689-4698.

Maxwell, C. V.; Davis, M. E.; Brown, D. C.; Bond, P. and Johnson, Z. B. 2003. Potential for fish meal analog as a replacement for fish meal in early-weaned pig diets. Arkansas Animal Science Department Report. p.153-157.

Lee, S. H.; Ingale, S. L.; Kim, J. S.; Kim, K. H.; Lokhande, A.; Kim, E. K.; Kwon, I. K.; Kim, Y. H. and Chae, B. J. 2014. Effects of dietary supplementation with Bacillus subtilis LS 1–2 fermentation biomass on growth performance, nutrient digestibility, cecal microflora and intestinal morphology of weanling pig. Animal Feed Science & Technology 188:102-110.

Liu, X.; Feng, J.; Xu, Z.; Lu,Y. and Liu, Y. 2007. The effects of fermented soybean meal on growth performance and immune characteristics in weaned piglets. Turkish Journal of Veterinary and Animal Sciences 31:341-345.

Liu, Y. 2015. Fatty acids, inflammation and intestinal health in pigs. Journal of Animal Science and Biotechnology 6:41.

Noh, H.S.; Ingale, S. L.; Lee, S. H.; Kim, K. H.; Kwon, I. K.; Kim, Y. H. and Chae, B. Jo. 2014. Effects of citrus pulp, fish by-product and *Bacillus subtilis* fermentation biomass on growth performance, nutrient digestibility, and fecal microflora of weanling pigs. Journal of Animal Science and Technology 56:10.1-10.7.

Wang, C. G.; Ding, X. M.; Bai, S. P. and Zhang, K. Y. 2009. Effects of fish meal and sodium butyrate on growth performance, gut development and glucagon-like peptide-2 secretion in weanling piglets. Journal of Life Sciences 3:3-15.

Yan, L.; Lim, S. U. and Kim, I. H. 2012. Effect of fermented chlorella supplementation on growth performance, nutrient digestibility, blood characteristics, fecal microbial and fecal noxious gas content in growing pigs. Asian-Australian Journal of Animal Science 25:1742-1747.

Zhang, K. Y.; Zhang, Y. Q.; Wang, Z. and Yu, Y. 2003. Effect of replacement of fish meal by poultry byproduct meal and meat and bone meal (regular, packer all beef and low ash renderer) in practical diets for newly weaned pigs on growth and feed utilization. National Renderers Association, Inc., USA.

Zinnerman, D. R. 1998. Nutritive value of some newly developed protein sources for early-weaned pig. p.101-109. In: Proc. Preconference symposia. The 8th World Conference on Animal Production. Seoul National University, Seoul, Korea.