

Evaluation of Recombinant Human Lactoferricin Culture as a Substitute for Antibiotic in Pig Starter Diets

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이유자돈사료에 항생제를 대체하기 위한 재조합 인간 락토페리신 컬처의 평가

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ABSTRACT

Sixty [(Duroc×Yorkshire)×Landrace] pigs (7.63±0.41kg average body weight and 25-d average age) were used in a 20-d growth assay to determine the effect of dietary recombinant human lactoferricin culture (RHLC) supplementation on growth performance, digestibility and plasma IgG concentration in weaning pigs. Dietary treatments included 1) Negative control (NC : without antibiotic), 2) Positive control (PC : NC diet + 0.1% chlortetracycline), 3) RHLC0.3 (NC diet + 0.3% RHLC), 4) RHLC0.5 (NC diet + 0.5% RHLC). No differences were found among treatments in average daily gain (P > 0.05). ADFI of pigs fed RHLC0.3 diet was higher than that of pigs fed PC diet (P < 0.05). However, pigs fed RHLC0.5 diet had improved gain/feed compared to pigs fed PC diet. Pigs fed PC and RHLC diets showed significantly increased dry matter digestibility compared to pigs fed NC diet (P < 0.05). There was no significant difference in plasma IgG concentrations (P > 0.05). The supplementation of RHLC in starter pig diets appears to be an alternative to antibiotics.

(Key words : Recombinant human lactoferricin culture; Antibiotic-free diet; Pigs)

I . INTRODUCTION

Weaning is stressful for pigs because of the adjustment from a liquid to a dry diet. This transition may alter the digestive tract's microbes, resulting in less desirable microflora that cause poor performance and diarrhea (van de Ligt et

al., 2002). Diarrhea in neonatal and postweaning pigs is a serious problem due to the trend towards large intensive herds and early weaning (at 14-21 days rather than 21-28 days of age). One of the most common causes of diarrhea is infection with enterotoxigenic *Escherichia coli* (ETEC) which is normally kept under control in

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the young pigs by passive immunity imparted by antibodies present in the milk. Early weaning has resulted in the removal of this protection and thereby has greatly increased the incidence of the disease. Also, ETEC 987P is the most common cause of diarrhea is pigs in chungnam province, Korea.

Control of intestinal pathogens has long been a goal of both livestock producer and microbiologist. Methods used to achieve this goal include addition of antibiotic to feed, acidification of drinking water, competitive exclusion of pathogens using probiotics and antimicrobial levels of minerals such as zinc oxide and copper sulfate. The most common method of repressing undesirable microbes has been the use of antibacterial agents. Recent interest has focused on nonantibiotic feed additions because of public concern over drug residue in meat products and development of antibiotic resistant pathogenic organisms (Kunin, 1993). A possible nonantibiotic additive for pig diets is active lactoferrin.

Lactoferrin is secreted by exocrine glands, such as the mammary, intestinal and salivary glands, and by neutrophils. It shows both direct and indirect antimicrobial action and anti-viral effects. The bacteriostatic effects of lactoferrin are usually attributed to its ability to bind environmental iron ion (Arnold et al., 1977; Reiter, 1978; Reiter, 1983). Also, lactoferrin has been shown to bind to the outer membrane of Gram-negative bacteria and it induces the release of lipopolysaccharides, leading ultimately to the destruction of these bacteria (Ellison et al., 1988; Ellison et al., 1990). Lactoferrin inhibits the growth of many Gram-positive and Gram-negative, as well as some species of mold and yeast.

We previously demonstrated the successful production of human lactoferricin using a recombinant *Pichia pastoris* expression system (Choi et al., 1999).

The purpose of this experiment was to examine recombinant human lactoferricin culture (RHLC)

as a substitute for antibiotics in the diets of weaned pigs.

II. MATERIALS AND METHODS

1. Animals and diets

Sixty [(Duroc×Yorkshire)×Landrace] pigs (7.63±0.41kg average body weight, 25-d average age and 21-d weaning age) were used in a 20-d growth assay to determine the effects of RHLC on growth performance, digestibility and serum IgG concentration in weaning pigs. The pigs were blocked by weight and assigned to treatments based on sex. There were three pigs per pen and five pens per treatment. Dietary treatments included 1) NC (Negative control : without antibiotic), 2) PC (Positive control : NC diet + 0.1% Chlortetracycline), 3) RHLC0.3 (NC diet + 0.3% RHLC, Easy-Bio System, Inc., Korea) and 4) RHLC0.5 (NC diet + 0.5% RHLC). NC diet (Table 1) was corn-dried whey-SBM based without antibiotic and formulated to contain 3,450 kcal ME/kg, 22.30% of crude protein, 1.50% of lysine, 0.42% of methionine, 0.90% of calcium and 0.80% of phosphorus. The diets were fed in meal form and formulated to be in excess of NRC (1998) recommendations for all nutrients. Chromic oxide was added (0.2% in the diet) as an indigestible marker to allow digestibility determinations. On d 15 of experiment, fecal samples were collected from two pigs per pen by rectal massage, pooled within pen, dried and ground.

2. Procedures

Pigs were allowed to consume feed and water on an *ad libitum* basis from a two holes feeder and nipple waterer. The pigs and feeder were weighed on d 10 and 20 to allow calculation of ADG, ADFI and gain/feed.

On day 0 of the experiment, pigs were dosed

Table 1. Formula and chemical composition of basal diet(as-fed basis)

Ingredients	%
Corn	36.53
Dried whey	25.00
Soybean meal (CP 48%)	16.40
Soy flour	12.50
Animal fat	5.00
Spray-dried blood meal	1.75
Tricalcium phosphate	1.67
Fish meal	0.28
Vitamin premix ¹⁾	0.25
Mineral premix ²⁾	0.15
L-lysine·HCl	0.13
DL-methionine	0.09
Antioxidant (Ethoxyquin 25%)	0.05
Chromic oxide ³⁾	0.20
Chemical composition ⁴⁾	
ME, kcal/kg	3,450
Crude protein (%)	22.30
Lysine (%)	1.50
Calcium (%)	0.90
Phosphorus (%)	0.80

¹⁾ Provided per kg of complete diet : 20,000 IU of vitamin A; 4,000 IU of vitamin D₃; 80 IU of vitamin E; 16 mg of vitamin K₃; 4 mg of thiamine; 20 mg of riboflavin; 6 mg of pyridoxine; 0.08 mg of vitamin B₁₂; 120 mg of niacin; 50 mg of Ca-pantothenate; 2 mg of folic acid and 0.08 mg of biotin.

²⁾ Provided per kg of complete diet : 140 mg of Cu; 179 mg of Zn; 12.5 mg of Mn; 0.5 mg of I; 0.25 mg of Co and 0.4 mg of Se.

³⁾ Used as an indigestible marker.

⁴⁾ Calculated value.

orally with *E. coli* 987P(1.0ml/dose; 1.2×10⁸/ml). ETEC 987P bacteria was obtained from *Escherichia Coli* Reference Centre, Pennsylvania State University, USA.

3. Laboratory and statistical analyses

Feed and feces were analyzed for DM and N concentrations(AOAC, 1995). Chromium was determined by UV absorption spectrophotometry (Shimadzu, UV-1201, Japan) and apparent digesti-

bilities of DM and N were calculated using the indirect-ratio method.

The concentration of IgG in the plasma was measured to investigate the effect of RHLC supplementation on immune system. Blood samples were collected via jugular vein into vacutainer tubes(Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) from four pigs in each treatment at the termination of the feeding trial. Blood samples were centrifuged at 2,000g for 30min. Plasma samples were taken and stored at -20°C until analyses for IgG. Plasma IgG concentration was determined by IgG Kit(Behring, Germany).

Statistical analyses were carried out to compare the means by Duncan's multiple range test (Duncan, 1955) using General Linear Model procedure of SAS(1996).

III. RESULTS AND DISCUSSION

The growth, feed intake and feed efficiency of pigs fed the experimental diets are presented in Table 2. For d 0 to 10, average daily feed intake(ADFI) of pigs fed RHLC0.3 diet was higher than that of pigs fed PC diet(P<0.05). However, pigs fed the RHLC0.5 diet had significantly improved gain/feed ratios(P<0.05) compared to pigs fed PC diet. For d 10 to 20, ADFI and gain/feed of pigs fed RHLC0.3 diet was higher than those of pigs fed PC diet (P<0.05). Through entire experimental period, ADFI of pigs fed RHLC0.3 diet was higher than that of pigs fed PC diet(P<0.05). Pigs fed RHLC0.5 diet had improved gain/feed compared to pigs fed PC diet.

Digestibility of dry matter and nitrogen in pigs fed experimental diet are presented in Table 3. Pigs fed PC and RHLC diets had significantly increased in dry matter digestibility compared to pigs fed NC diet(P<0.05).

Plasma IgG concentration of pigs fed exper-

Table 2. Effects of dietary recombinant human lactoferrin culture on growth performance in weaned pigs¹⁾

Item	NC ²⁾	PC ²⁾	RHLC0.3 ²⁾	RHLC0.5 ²⁾	SE ³⁾
0-10 days					
Average daily gain (g)	243	282	328	351	31
Average daily feed intake (g)	401 ^b	398 ^b	459 ^a	410 ^b	21
Gain/feed	0.61 ^c	0.71 ^b	0.71 ^b	0.86 ^a	0.05
10-20 days					
Average daily gain (g)	472	487	565	514	33
Average daily feed intake (g)	734 ^b	761 ^b	803 ^a	769 ^b	26
Gain/feed	0.64 ^b	0.64 ^b	0.70 ^a	0.67 ^{ab}	0.02
0-20 days					
Average daily gain (g)	358	385	447	433	26
Average daily feed intake (g)	568 ^b	579 ^b	631 ^a	590 ^b	21
Gain/feed	0.63 ^b	0.67 ^b	0.71 ^{ab}	0.73 ^a	0.02

¹⁾ Sixty pigs with an average initial body weight of 7.63±0.41kg (SD).

²⁾ Abbreviated NC, negative control added not antibiotic; PC, positive control added antibiotic; RHLC0.3, added 0.3% of recombinant human lactoferrin culture; RHLC0.5, added 0.5% of recombinant human lactoferrin culture.

³⁾ Pooled standard error.

^{abc} Means in the same row with different superscripts differ significantly (P<0.05).

Table 3. Effects of dietary recombinant human lactoferrin culture on nutrient digestibility and plasma IgG concentration in weaned pigs¹⁾

Item	NC ²⁾	PC ²⁾	RHLC0.3 ²⁾	RHLC0.5 ²⁾	SE ³⁾
Dry matter (%)	82.02 ^b	85.69 ^a	87.08 ^a	83.57 ^a	0.35
Nitrogen (%)	81.62	83.69	85.80	83.21	0.43
Plasma IgG (mg/dL)	352.8	430.7	468.1	355.9	48.9

¹⁾ Sixty pigs with an average initial body weight of 7.63±0.41kg (SD).

²⁾ Abbreviated NC, negative control added not antibiotic; PC, positive control added antibiotic; RHLC0.3, added 0.3% of recombinant human lactoferrin culture; RHLC0.5, added 0.5% of recombinant human lactoferrin culture.

³⁾ Pooled standard error.

^{ab} Means in the same row with different superscripts differ significantly (P<0.05).

imental diets are presented in Table 3. There was no significant difference in IgG concentrations of plasma (P>0.05). The conclusion of RHLC in starter pig diets appears to be a viable alternative to antibiotics.

Lactoferrin is an iron-binding protein found in human mucosal secretions as well as the spe-

cific granules of polymorphonuclear leukocytes. A variety of functions have been ascribed to the protein and proposed for lactoferrin included protection against microbial infections (Spik et al., 1978; Nemet and Simonovits, 1985; Ellison and Giehl, 1991; Yamauchi et al., 1993), regulation of immune function (Crouch et al., 1992; Machnicki

et al., 1993), regulation of myelopoiesis (Sawatzki and Rich, 1989; Hangoc et al., 1991), cellular growth promotion (Nichols et al., 1987; Nichols et al., 1990), regulation of intestinal iron absorption (Davidson and Lonnerdal, 1989; Iyer and Lonnerdal, 1993) and regulation of transcription (Penco et al., 1995; He and Furmanski, 1995).

Bacteriostatic effects of lactoferrin are usually attributed to its ability to bind environmental iron ions. This protein inhibits the growth of many Gram-positive and Gram-negative bacteria, as well as some species of mold and yeast. Aguila et al. (2001) demonstrated that therapeutic approaches based on the use of ferrochelating agents such as lactoferrin combined with antimicrobial drugs may help to counteract the reduced efficacy of current antibiotics. Lee et al. (1998) evaluated the ability of lactoferrin to protect germ free, colostrums-deprived piglets against a lethal intravenous challenge with endotoxin ; it was shown that prefeeding with lactoferrin resulted in a significant decrease in piglet mortality compared to feeding with bovine serum albumin. The current study demonstrates that RHLC improves the feed efficiency of piglets fed antibiotic-free diets.

IV. SUMMARY

개시시 체중 7.63 ± 0.41 kg, 3월 교잡종 이유자돈(25일령) 60두를 공시하여, 이유자돈 사료내 재조합 인간 락토페리신 컬처(RHLC ; recombinant human lactoferricin culture)의 급여가 성장, 영양소 소화율 및 혈장내 면역글로블린 G 농도에 미치는 영향을 평가하기 위하여 20일간 사양시험을 실시하였다. 시험설계는 1) 항생제 무첨가구(NC), 2) 항생제 첨가구(PC; NC 사료 + 0.1% chlortetracycline), 3) 항생제 무첨가구 사료에 RHLC를 0.3% 첨가한 처리구(RHLC0.3; NC 사료 + 0.3% RHLC), 4) 항생제 무첨가구 사료에 RHLC를 0.5% 첨가한 처리구(RHLC0.5; NC 사료 + 0.5% RHLC)로 4개 처리로 하였다. 총 사양시험 기간동안, 일당증체량에 있어서는 항생제 첨가구와 비교하여 RHLC

의 첨가수준이 증가함에 따라 높아지는 경향을 보였으나, 표준오차가 크기 때문에 유의적인 차이는 보이지 않았다. 일당사료섭취량에 있어서는 PC 처리구와 비교하여 RHLC0.3 처리구가 유의적으로 높았으며($P < 0.05$), 사료효율에 있어서는 PC 처리구와 비교하여 RHLC0.5 처리구가 가장 좋은 것으로 나타났다($P < 0.05$). 영양소 소화율에 있어서는 NC 처리구와 비교하여 PC, RHLC0.3 및 RHLC0.5 처리구가 유의적으로 높게 평가되었으며($P < 0.05$), 혈장내 면역글로블린 G 농도는 처리구간에 유의적인 차이를 보이지 않았다. 결론적으로, 이유자돈에 있어 재조합 인간 락토페리신 컬처가 항생제를 대체할 가능성이 있는 것으로 사료된다.

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