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## NEONATAL FC RECEPTOR mRNA EXPRESSION IN GASTROINTESTINAL TISSUES FROM PIGS FED MEAL OR PELLETTED DIETS WITH OR WITHOUT IRRADIATED AND NON-IRRADIATED SPRAY-DRIED ANIMAL PLASMA

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### Summary

The neonatal Fc receptor (FcRn) participates in intracellular trafficking of IgG and the maintenance of circulating IgG. The relationship between the FcRn and IgG may also augment host defense immunosurveillance. The current studies evaluated FcRn mRNA from intestinal tissues in fetal pigs and FcRn mRNA in weaned pigs fed meal or pelleted diets with or without irradiated or non-irradiated spray-dried animal plasma.

In Exp. 1, fetal pigs were obtained at d 55 and 70 of gestation (n = 5 fetuses/gestational age) and total RNA was isolated from intestinal tissues for quantitative real-time PCR (qPCR) to determine mRNA for FcRn. The FcRn transcripts were observed in all samples, and greater levels of FcRn mRNA were observed in d 55 fetuses compared to d 70 fetuses.

In Exp. 2, weaned pigs were used in an 11-d growth assay to determine the effects of feeding meal and pelleted diets with irradiated or non-irradiated spray-dried animal plasma (AP 920) on FcRn expression in intestinal tissues.

Pigs were blocked by weight and randomly allotted in a 2 × 2 factorial to one of

four dietary treatments. Main effects were diet form (meal or pellet) and either irradiated or non-irradiated spray-dried animal plasma. Jejunal, ileal, and cecal tissues were collected from 24 pigs at the conclusion of the growth assay. Total RNA was isolated to quantify relative mRNA expression of FcRn. The FcRn mRNA transcripts were observed in all tissues. The FcRn mRNA was more abundant ( $P < 0.02$ ) in pigs fed the non-irradiated plasma compared with the pigs fed irradiated plasma. The FcRn mRNA was more abundant ( $P < 0.05$ ) in pigs fed the meal diets compared with the pigs fed pelleted diets.

In conclusion, these data suggest that fetal and weanling pig tissues have FcRn mRNA present in the jejunal, ileal, and cecal sections of the small intestine. These data also indicate that FcRn varies with age in pigs. Diet form (meal or pellet) and irradiation of spray-dried animal plasma affects the expression of FcRn in weanling pigs.

(Key words: feed manufacturing, Fc receptor, gestation.)

### Introduction

The classic role of the neonatal Fc receptor (FcRn) is to transport IgG from milk across intestinal epithelial cells in newborns. The re-

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ceptor also has been implicated in extending the half-life of circulating IgG. The neonatal Fc receptor, FcRn, is a heterologous macromolecule, structurally similar to MHC class-I, consisting of a triplet of Ig-like  $\alpha$  chains associated with  $\beta$ -2-microglobulin. The neonatal Fc receptor participates in intracellular trafficking of IgG and the maintenance of circulating IgG. Evidence from the human and murine experiments suggests that the relationship between the FcRn and IgG may augment host defense immunosurveillance. FcRn was detected by immunostaining on the apical surface of human fetal small intestine and was found to be equally distributed among stomach, ileal, and colonic epithelium. However, the intestinal expression of FcRn has not been evaluated in domestic pigs. Therefore, the objective of Exp. 1 was to evaluate the presence and relative abundance of FcRn mRNA from intestinal tissue in fetal pigs. Experiment 2 further evaluated the presence and relative abundance of FcRn mRNA in intestinal tissues of weaned pigs fed meal or pelleted diets with or without irradiated or non-irradiated spray-dried animal plasma.

## Materials and Methods

**Experiment 1.** Fetal pigs were surgically removed from three sows at d 55 and three sows at d 70 of gestation with one or two fetuses collected per sow (5 fetuses/gestational age). Intestinal tissues were collected from fetuses and total RNA was isolated for quantitative real-time PCR (qPCR) to determine expression of FcRn mRNA.

**Experiment 2.** A total of 48 pigs (PIC; Initial BW 5.2 kg) were used in an 11-d growth assay. Pigs were blocked by weight, and randomly allotted in a  $2 \times 2$  factorial to one of four dietary treatments (Table 1). Main effects were diet form (meal or pellet) and either irradiated or non-irradiated spray-dried animal plasma. Twenty-four pigs were randomly selected and euthanized on d 11. An incision was made down the abdominal mid-

line, and the ileocecal junction was immediately located. The jejunal, ileal, and cecal sections were immediately clamped off and digestive contents and section tissues samples were collected in 1.5 mL microcentrifuge tubes. All samples were snap frozen in liquid nitrogen and remained frozen at  $-80^{\circ}\text{C}$  until assayed. Tissues were homogenized and genomic DNA was extracted from both tissues and contents using the MO BIO Laboratories, Inc. UltraClean<sup>TM</sup> Fecal DNA Kit (Carlsbad, California). The isolated DNA was used for quantitative real-time PCR (qPCR) to determine expression of FcRn mRNA.

Statistical analysis was performed using the MIXED procedures in SAS. Pig was used as the experimental unit.

## Results and Discussion

**Experiment 1.** The FcRn transcripts were observed in all fetuses (Figure 1). Relative abundance of FcRn was lower in the d 70 fetuses compared to the d 55 fetuses. This is an indication that the FcRn may be more important earlier in the life of the pig, and may decrease rapidly as the pig ages. Due to the small number of observations in the study it is difficult to draw a strong conclusion about the differences in expression between d 70 and 55 fetuses. However, this data confirms the presence of the FcRn in the gastrointestinal tract of pigs.

**Experiment 2.** The FcRn transcripts were observed in all tissue samples (Figure 2), indicating the presence of the FcRn mRNA in pigs. The FcRn mRNA was more abundant ( $P < 0.02$ ) in pigs fed the non-irradiated spray-dried animal plasma compared with the pigs fed irradiated animal plasma. The FcRn mRNA was more abundant ( $P < 0.05$ ) in pigs fed the meal diets compared with the pigs fed pelleted diets. This may indicate that pigs fed the meal diet and the diet containing non-irradiated spray-dried animal plasma may have a greater need for IgG absorption for

immune status or defense mechanism. However, it is not clear if an increase in FcRn mRNA expression in pigs alters IgG status as mRNA presence is not a direct indicator of the function protein.

These data suggest that fetal and weanling pig tissues have FcRn mRNA present in the jejunal, ileal, and cecal sections of the small intestine. These data also may imply that FcRn mRNA may vary with age, but more

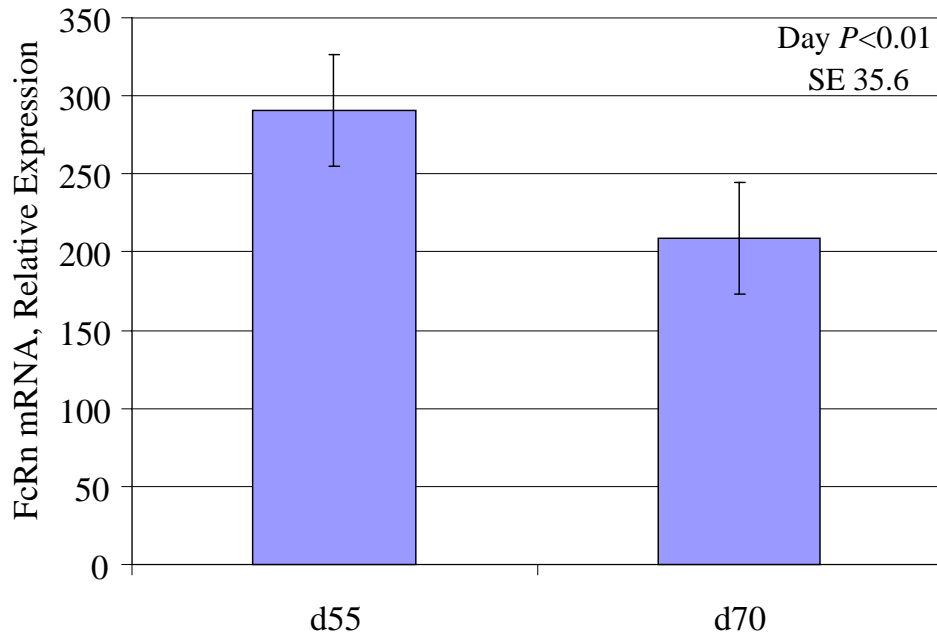
research is required to determine if this is a true response. Diet form (meal or pellet) and irradiation of spray-dried animal plasma affects the expression of FcRn mRNA in weanling pigs. These data suggest that dietary manipulation may alter the receptor mRNA expression and may lead to changes in IgG absorption status of the pig. However, additional research would be required to determine changes in IgG status, as an indication of changes or enhanced immune function.

**Table 1. Composition of Diets, As-Fed Basis (Experiment 2)**

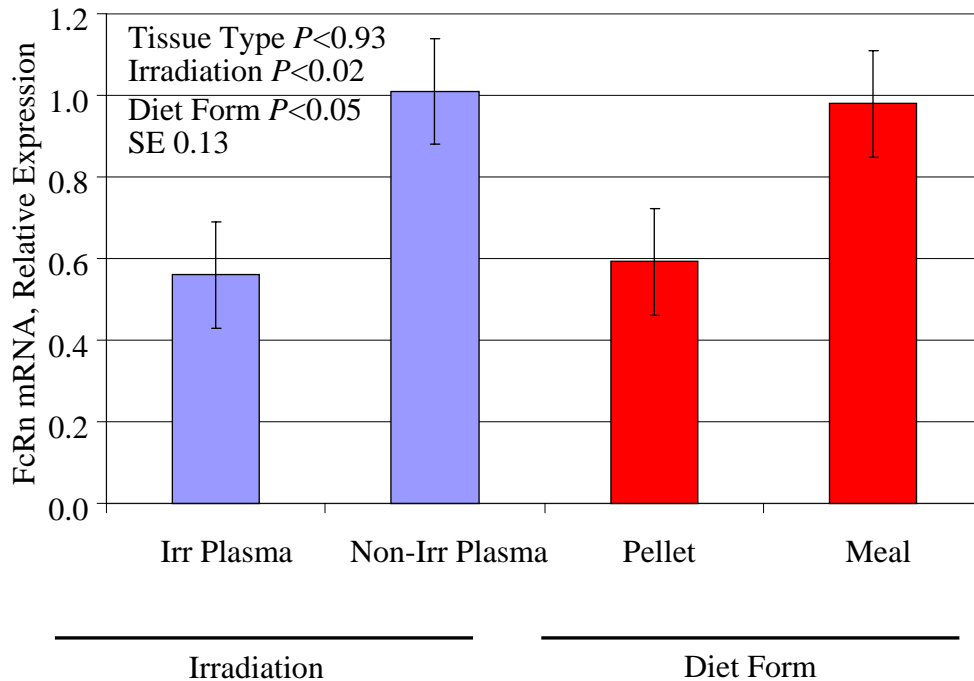
Item, %	d 0 to 11 <sup>a</sup>
Corn	44.01
Soybean meal (46.5% CP)	19.40
Spray dried whey	20.00
Spray dried animal plasma	5.00
Menhaden fish meal	5.00
Soy oil	3.00
Monocalcium P (21% P)	0.75
Limestone	0.65
Salt	0.25
Vitamin premix	0.25
Trace mineral premix	0.15
Antibiotic <sup>b</sup>	0.70
Zinc oxide	0.38
L-Threonine	0.08
L-Lysine HCl	0.23
DL-Methionine	0.15
	100.00
Calculated analysis	
Total lysine, %	1.50
ME, kcal/lb	1,552
Protein, %	22.6
Ca, %	0.88
P, %	0.80
Available P, %	0.57
Lysine:calorie ratio, g/Mcal	4.38

<sup>a</sup>The phase 1 (d 0 to 11) diet was feed in either meal or pelleted form with irradiated spray dried animal plasma or non-irradiated spray dried animal plasma.

<sup>b</sup>Provided 140g Neomycin sulfate and 140g Oxytetracycline HCl per ton of feed.



**Figure 1. The Relative Expression of FcRn mRNA in Fetal Pig Intestinal Tissues Collected at D 55 and D 70 of Gestation.** The Fc receptor transcripts were observed in all fetuses. Relative abundance of FcRn was lower in the d 70 fetuses compared to the d 55 fetuses.



**Figure 2. The Effects of Plasma Irradiation (Irr) and Diet Form on the Relative Abundance of the FcRn in Weanling Pigs.** The FcRn mRNA was more abundant ( $P < 0.02$ ) in pigs fed the non-irradiated plasma compared with the pigs fed irradiated plasma. The FcRn mRNA was more abundant ( $P < 0.05$ ) in pigs fed the meal diets compared with the pigs fed pelleted diets.