RESEARCH REPORT
ANIMAL SCIENCE

Title: Effect of pregnant gilt zinc and lipid supplementation on neonatal piglet brain myelination and preweaning mortality, **NPB #09-024**

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Industry Summary

The effect of dietary supplementation of pregnant gilts with Gromega lipid supplement (JBSUnited feeds), zinc sulfate, or the two combined on development of specific regions of newborn piglet brains was examined in this experiment. The relationship between development of brain regions, preweaning mortality and neonatal nursing ability was also explored. A simple, rapid and inexpensive assay, the “immunocrit”, was developed and validated as a simple indicator of newborn piglet nursing ability. Results of the experiment indicated a significant but weak association between the degree of development of the brain stem and immunocrit measurements. Combined Gromega and zinc supplementation of gilts increased development of this same region of the piglet brain, but did not improve immunocrit or preweaning survival. The immunocrit assay will be a valuable tool to assess neonatal piglet nursing ability in future experiments to improve this trait in newborn piglets. Although this experiment suggests that combined Gromega and zinc supplementation may be beneficial to neonatal piglet brain development, further research is needed to determine whether this improved brain development results in improvement in preweaning survival of piglets.

Keywords

Preweaning mortality, myelin, brain, immunocrit, zinc, Gromega

Scientific Abstract

Preweaning mortality of piglets ranges from 10 to 20% and appears to be increasing as sows are selected for increased litter size. Myelination of the brain is reduced in low birth weight piglets and it has been hypothesized that this may contribute to poor preweaning survival, especially in larger litters where low birth weight piglets occur at greater frequency. The effect of Gromega and/or zinc supplementation of gestating gilts on myelination of the brain stem, cerebellum and spinal

cord of the smallest piglets in each litter was tested by measuring myelin basic protein or myelin cholesterol, glucocerebrosides, phosphatidylethanolamine, phosphatidylcholine and sphingomyelin in isolated myelin membranes from these brain regions. Gilts were mated and fed either a control diet, a diet supplemented with 1.09% Gromega, a diet supplemented with 0.07% Zinc Sulfate or a diet supplemented with both Gromega and Zinc Sulfate from day 80 of gestation until farrowing. On day 1 after farrowing, piglets were weighed and the smallest piglet in each litter was sacrificed and blood was collected. Brain, cerebellum, brain stem, full and empty stomach (to calculate stomach content weight) and heart weights were recorded. Brain stem, cerebellum and spinal cord tissues were collected and myelin membranes were isolated. Myelin basic proteins and myelin lipids were measured using SDS-PAGE and Thin layer chromatography, respectively, followed by densitometry. Blood was assayed for immunoglobulin G using a new rapid assay, the “immunocrit”, developed for this purpose. Results indicated a weak correlation ($r = 0.23$, $P < 0.05$) between immunocrit and brain stem high molecular weight myelin basic protein, suggesting that brain stem myelination may contribute to nursing ability. There was also a Gromega by zinc supplementation interaction ($P < 0.05$) on brain stem high molecular weight myelin basic protein. Despite this, there were no effects of dietary supplementation on weight of the stomach contents, immunocrit or preweaning survival and there was no relationship between any myelin measurement and preweaning survival.

Introduction

Between 10 and 20% of piglets born alive die before weaning (Fahmy and Bernard,1971; Fahmy et al., 1978), and the majority of this loss occurs within the first 2 or 3 days of life. As swine producers have selected for increased litter size, preweaning mortality has also increased, primarily due the suppression of newborn piglet birth weight, which is a known factor contributing to preweaning mortality (Bereskin et al., 1973; Tuscherer et al., 2000; Arango et al., 2006). The temporal pattern of preweaning mortality suggests that a useful strategy would be to focus efforts on improving aspects of physiology of low birth weight piglets during late gestation (Van der Lende, 2001), but it remains unclear what aspects of the low birth weight newborn piglet predispose it to high mortality.

One aspect that has been proposed to contribute to poor preweaning survival is reduced brain myelination in small piglets (Dickerson et al. 1971; Pond et al., 2000). Myelination is the encasing of specific neurons with multiple layers of specialized plasma membranes, composed primarily of lipids (Boiko and Winkler, 2006). Myelination increases the speed of nerve impulses along axons, and poor brain myelination could contribute to increased preweaning mortality by decreasing both reflex speed and coordination of movement of low birth weight piglets (Deber and Reynolds, 1991; Fields, 2008). We recently confirmed that the brain regions of small fetuses are less myelinated during late gestation compared to large littermates (Vallet and Miles, 2010) by measuring myelin basic protein and myelin lipid in myelin membranes isolated from specific brain regions on day 93, 100 and 110 of gestation and demonstrated that the greatest difference in myelination between large and small fetuses occurred within the brain stem. Previous research in rodents suggests that supplementation of gestation diets with essential fatty acids improved brain myelination (Berkow and Campagnoni, 1983; Salvati et al., 1996; Yehuda et al., 2005). In addition, zinc is known to participate with myelin basic proteins in the myelin compaction process (Kursula, 2008), which is required for the efficient wrapping of myelin membranes around nerve axons. Thus, we hypothesized that decreased brain myelination may contribute to reduced ability of low birth weight piglets to perform the essential functions of neonatal life and therefore to increased preweaning mortality. We further hypothesized that essential lipid supplementation (in the form of Gromega supplement from JBSUnited Inc.) and/or zinc sulfate supplementation would improve neonatal piglet brain myelination, neonatal piglet function, and preweaning survival.

Few simple methods exist to evaluate the ability of newborn piglets to perform the essential functions of life. Arguably, the most important of these abilities is the ability of the piglet to acquire sufficient colostrum from the dam, which has been reported to be essential to provide the dietary energy required by the piglet to sustain life beyond the first 24 hours (Le Dividich et al., 1994; Noblet et al., 1997). Measurement of colostrum requires multiple weight measurements of piglets and attendance during farrowing, and is therefore not very convenient to measure. Because ingestion of colostrum by piglets results in significant concentrations of immunoglobulin G (IgG) in neonatal piglet serum by day 1 of age (i.e. passive transfer), piglet serum IgG concentrations on day 1 likely reflect ingestion of colostrum since birth, and are therefore likely to reflect neonatal piglet nursing ability. Yaguchi et al. (1980) reported an assay for piglet serum IgG based on ammonium sulfate precipitation and measurement of absorbance. This assay suffers from high background absorbance of samples, necessitating performing background analysis on every sample, which is time consuming. We experimented with the use of a microcapillary tube to separate and measure the IgG precipitated using ammonium sulfate. Here we provide validation of this procedure, termed an “immunocrit”, for the measurement of piglet serum IgG, confirm that it is correlated with the weight of piglet stomach contents on day 1, and use it to assess the effects of treatment and associations with measurements of brain myelination.

Objectives

1. Develop and validate the immunocrit procedure as a measure of serum IgG.

2. Determine whether lipid and/or zinc supplementation of sow diets will affect cerebellum, brain stem and spinal cord myelination in newborn piglets.

3. Determine whether improved brain myelination is associated with decreased stillbirth and preweaning mortality of piglets.

Materials and Methods

Landrace, Duroc, and York crossbred gilts from the USMARC BX population that farrowed in the 200903 and 200904 farrowing seasons were used in this experiment. Gilts were heat checked beginning about 170 days of age and were mated at standing estrus at about 250 days of age using artificial insemination. From day 80 of gestation until farrowing, pregnant gilts were fed one of four dietary treatments: 1) a control feed consisting of the standard lactating sow feed at USMARC (consisting of 72.6% ground corn, 22.9% Soybean meal, 1.6% Dicalcium Phosphate, 0.7% limestone, 0.5% NaCl, 0.2% Vitamin Premix 11,0.2%Trace mineral H, 0.2% choline chloride, 0.13% Tylan 40, and 1% soybean oil), 2) Sow feed supplemented with 1.09% Gromega lipid supplement (JBS United feeds, Sheridan IN), 3) Sow feed supplemented with .07% zinc sulfate and 4) Sow feed supplemented with Gromega and zinc sulfate combined. Gilts were moved to the farrowing house at approximately 110 days of gestation. Farrowings were video recorded to determine individual piglet birth intervals for each piglet. On day 1 after farrowing, piglets were weighed and the smallest living piglet (by weight) in each litter was sacrificed after rendering the piglet unconscious using CO_2. A section of spinal cord just above the hip bone, blood, stomach, heart and brain were collected. The stomach was weighed, emptied, and reweighed to determine the weight of stomach contents. The heart and brain were weighed and then the cerebellum and brain stem were dissected and weighed. A portion of the cerebellum, brain stem and spinal cord was homogenized in 1 M sucrose buffer, and the homogenates were frozen at -20 C until further processed. Blood was allowed to clot, and serum was collected and frozen at -20 C.

Serum samples were used to assess the passive transfer of immunoglobulin from the sow to the piglet. We developed a simple assay, the “immunocrit” for this purpose. An aliquot (50 µl) of serum was mixed with 50 µl 40% ammonium sulfate, loaded into a hematocrit microcapillary tube, centrifuged for 5 minutes in a hematocrit centrifuge, and the ratio of the length of the precipitated IgG in the tube to the length of the sample in the tube was used as a quantitative measure of serum IgG. To validate the immunocrit assay, IgG was measured in two alternative ways and results of the immunocrit assay were compared. Serum samples were diluted 1:30 in 10 mM Tris, pH 8, 1 M NaCl, 1% Triton buffer (wash buffer) and 100 µl aliquots of the diluted samples were incubated for 24 h with 20 µl of a 10% solution of Protein A-Sepharose (Sigma-Aldrich, St. Louis, MO) in wash buffer. Samples were centrifuged and the pellet was washed 4 times in wash buffer and then a final time in 10 mM Tris, pH 8.0 to remove the Triton. Pellets were mixed with 100 µl gel loading buffer (Buhi et al., 1989), boiled for 5 minutes and subjected to SDS-PAGE on 10 % acrylamide gels. Gels were stained with coomassie, and the band corresponding to the high molecular weight band of IgG was quantified by densitometry. Serum samples were also measured for IgG using the previously described assay of Yaguchi et al. (1980).

To measure myelin in the brain and spinal cord tissues, 0.5 ml aliquots of tissue homogenates in 1 M Sucrose were centrifuged at 10,000 x g in a microcentrifuge and the supernatants retained. Due to their density, myelin membranes float in 1 M sucrose (Horrocks, 1967). The supernatant was then diluted with 4.5 ml 10 mM Tris, pH 8.0 and centrifuged again at 3000 x g to pellet the myelin membranes. To assess myelin basic protein content of the isolated membranes, the pellet was subsequently diluted in 1 ml SDS-gel loading buffer (Buhi et al., 1989) and boiled for 5 minutes. Then, 100 ul of each sample was electrophoresed on 12.5% acrylamide SDS-PAGE gels. Gels were stained with coomassie, imaged, and the density of the bands corresponding to high molecular weight and low molecular weight myelin basic proteins were determined using 2D-Advanced software (Nonlinear Dynamics LTD, Durham, NC). To assess lipid components in myelin membranes, further 0.5 ml aliquots of homogenate were treated similarly to isolate myelin membranes, and the pelleted membranes were extracted in chloroform-methanol (2:1) and subjected to thin layer chromatography (TLC) using a mobile phase of chloroform, methanol and 15 N ammonium hydroxide (16.25:6.25:1; Horrocks, 1968). Chromatographs were stained with amido black to visualize lipids and densitometry measurements were made on the resulting 6 major bands, corresponding to cholesterol, glucocerebrosides 1 and 2, phosphatidylethanolamine, phosphatidylcholine and sphingomyelin (Horrocks, 1968; Vallet unpublished results).

The numbers of mummies, piglets born alive and stillborn were recorded for each litter. Video recordings were observed to determine the time of birth of each piglet. The date and reason for all piglet deaths was recorded for the remaining live piglets in each litter and each litter was weaned at approximately 17 days. All piglets alive at weaning were weighed.

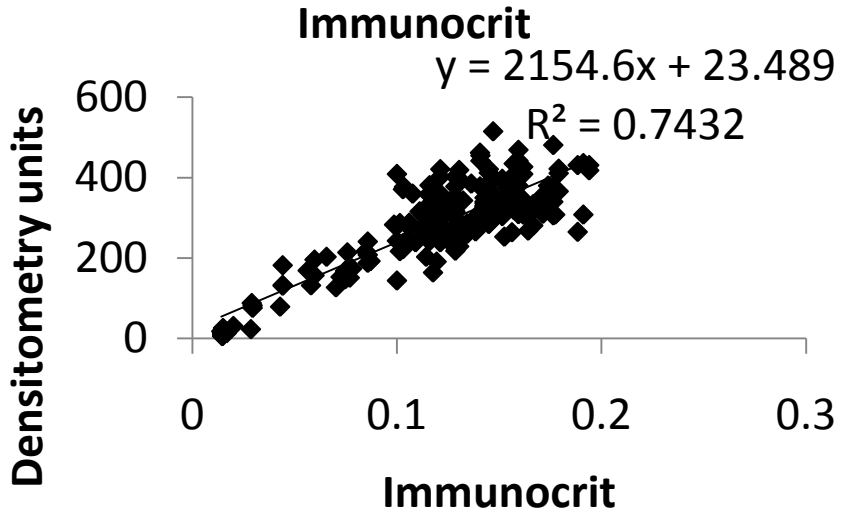
Number of piglets born alive and stillborn; birth weight of the smallest piglet in the litter; litter average birth weight; brain, heart, cerebellum and brain stem weights; densitometry data for myelin membrane high and low molecular weight myelin basic protein from brain stem, cerebellum and spinal cord tissue; myelin membrane lipid components from TLC analysis of brain stem, cerebellum and spinal cord tissue; weight of the stomach contents; immunocrit values; litter preweaning mortality and litter average weaning weight were analyzed using PROC GLM of SAS. A 2x2x2 factorial ANOVA model was used that included effects of season, Gromega supplementation, Zinc supplementation, and the Gromega by Zinc interaction. A further analysis of brain, heart, cerebellum and brain stem weights was conducted with the same model above along with inclusion of birthweight of the piglet as a covariate. A further analysis of the myelin basic protein and myelin lipid TLC data was conducted using the same model above with the inclusion of myelin cholesterol as a covariate. Simple correlation analyses were performed between all traits measured in this experiment. The associations between the high and low molecular weight myelin basic proteins in the brain stem, cerebellum and spinal cord with immunocrit measurements were further explored using regression analysis after

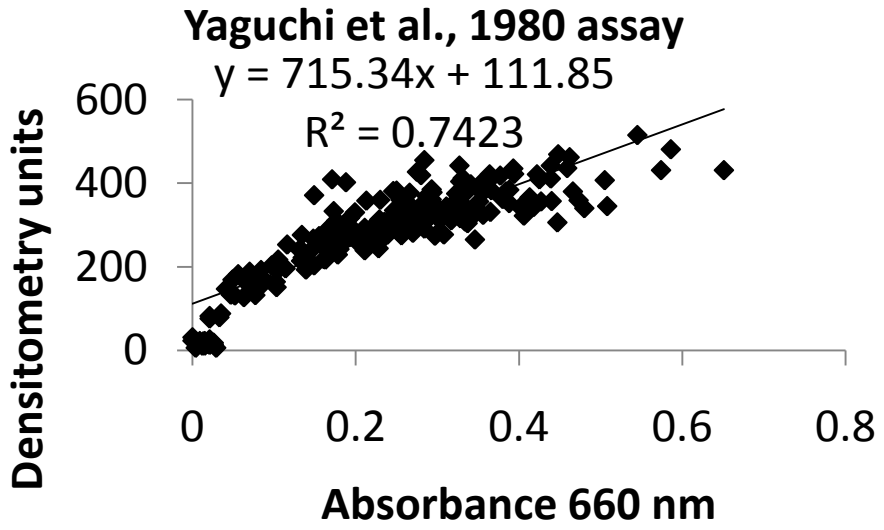
fitting the 2x2x2 ANOVA model described previously. To analyze birth interval data, the position of each piglet in the litter (birth position) was converted to a proportion of the litter farrowed by dividing the birth position by the total litter size. Proportion of the litter farrowed values were then grouped into 10 categories: (1) 0.1 – proportion of the litter farrowed from 0 to 0.15, (2) 0.2 – proportion of the litter farrowed from 0.15 to 0.25, (3) 0.3 – proportion of the litter farrowed from 0.25 to 0.35, (4) 0.4 – proportion of the litter farrowed from 0.35 to 0.45, (5) 0.5 – proportion of the litter farrowed from 0.45 to 0.55, (6) 0.6 – proportion of the litter farrowed from 0.55 to 0.65, (7) 0.7 – proportion of the litter farrowed from 0.65 to 0.75, (8) 0.8 – proportion of the litter farrowed from 0.75 to 0.85, (9) 0.9 – proportion of the litter farrowed from 0.85 to 0.95, (10) 1 – proportion of the litter farrowed from 0.95 to 1 (this category contains all the piglets that were born in the last position in each litter). Then, log transformed (to control heterogeneity of variance between categories) birth intervals were analyzed using a model that included the effects of season, Gromega, zinc, the Gromega by zinc interaction, proportion of the litter farrowed category, the Gromega by proportion of the litter farrowed interaction, the zinc by proportion of the litter farrowed interaction, and the Gromega by zinc by proportion of the litter farrowed interaction. Litter size was included as a covariate in the model and gilt within the Gromega by zinc interaction was included as a random effect. There were only 19 observations in the 0.1 proportion of the litter farrowed category distributed among the four treatment groups and variances were exceptionally high, so these birth interval data were reanalyzed using the same model described previously but with these data omitted to clarify significant treatment interactions with other proportion of the litter farrowed categories.

Results

Comparison of the results of the immunocrit and the IgG assay of Yaguchi et al. (1980) with results of IgG measurement using SDS-PAGE and densitometry are illustrated in Figure 1. Both assays were equally correlated ($r = 0.86$ for both) with the results of SDS-PAGE analysis of IgG, indicating that both were equally able to measure serum IgG. Thus, the immunocrit is as valid a measure of piglet serum IgG as the assay of Yaguchi et al. (1980).

Figure 1: The top graph illustrates the relationship between immunocrit measurements of IgG in serum compared to results obtained using Protein A Sepharose precipitation of serum IgG followed by SDS-PAGE and densitometry. The bottom graph illustrates the relationship between results of the absorbance based assay of Yaguchi et al. (1980) and SDS-PAGE analysis of IgG. The immunocrit and Yaguchi et al. (1980) assays were equally correlated ($r=0.86$, $P<0.01$) with the SDS-PAGE densitometry results, indicating that the immunocrit is as valid a measure of serum IgG as the Yaguchi et al. (1980) assay.





The effects of treatments on the traits measured in this experiment are summarized in Table 1. There were few significant treatment effects. However, birth weight and heart weight of the smallest piglet in each litter was significantly greater ($P < 0.05$) after Gromega supplementation, regardless of zinc supplementation. There was a significant Gromega by zinc interaction for cerebellum weight, which appeared to be due to a reduction of cerebellum weight after Gromega supplementation, but not when Gromega was supplemented along with zinc. The number of stillborn piglets was significantly greater ($P < 0.05$) after Gromega treatment, but the number born alive was not significantly affected. Turning to the myelin measurements, a Gromega by zinc interaction was observed ($p < 0.05$) for brain stem high molecular weight myelin basic protein, which appeared to result from a significant increase in high molecular weight myelin basic protein when Gromega and Zinc supplementations were combined, compared to the untreated control or either supplementation alone. A Gromega by zinc interaction was also observed ($P = 0.01$) for spinal cord high molecular weight myelin basic protein, but in this case appeared to be due to a reduction in high molecular weight myelin basic protein in spinal cord when gilts were supplemented with Gromega, but not when Gromega was combined with zinc supplementation. The only myelin lipid affected by treatment was brain stem myelin glucocerebroside 1, which was increased ($P < 0.05$) by zinc supplementation, all other myelin lipids in the neural tissues examined were unaffected by treatment. Stomach contents, immunocrit values, and preweaning mortality were also not affected by treatments.

Table 1. Least squares means (± SEM) for various traits measured in this experiment after control, Gromega supplementation, zinc sulfate supplementation or the two supplements combined are summarized.

Trait	Control	Gromega (G)	Zinc (Z)	G + Z
Number of gilts	52	59	56	53
Total born (inc. mummies)	10.3 ± 0.4	10.4 ± 0.4	11.1 ± 0.4	10.5 ± 0.4
Born alive	9.5 ± 0.4	9.4 ± 0.4	10.2 ± 0.4	9.2 ± 0.4
Stillborn[a]	0.5 ± 0.2	0.9 ± 0.2	0.6 ± 0.2	1.0 ± 0.2
Weaned	7.8 ± 0.4	7.6 ± 0.3	8.3 ± 0.4	7.5 ± 0.4
Smallest piglet birth weight, kg[a]	1.16 ± 0.04	1.23 ± 0.04	1.12 ± 0.04	1.23 ± 0.04
Average birth weight, kg	1.48 ± 0.03	1.53 ± 0.03	1.47 ± 0.03	1.49 ± 0.03
Average wean weight, kg	5.4 ± 0.1	5.5 ± 0.1	5.3 ± 0.1	5.4 ± 0.1
Stomach contents, g	33 ± 3	32 ± 3	30 ± 3	33 ± 3
Immunocrit	0.125 ± 0.006	0.119 ± 0.006	0.120 ± 0.006	0.119 ± 0.006
Preweaning mortality, %	7.4 ± 1.7	8.3 ± 1.6	8.4 ± 1.6	9.0 ± 1.7
Brain weight, g	30.8 ± 0.3	30.3 ± 0.3	30.2 ± 0.3	30.6 ± 0.3
Cerebellum weight, g[b]	3.21 ± 0.04	3.03 ± 0.04	3.09 ± 0.04	3.16 ± 0.04
Brain stem weight, g	1.43 ± 0.02	1.39 ± 0.02	1.37 ± 0.02	1.39 ± 0.02
Heart weight, g[a]	8.6 ± 0.4	9.7 ± 0.4	8.7 ± 0.4	9.4 ± 0.4
Brain stem HMWMBP[c]	339 ± 10	339 ± 9	324 ± 9	361 ± 10
Brain stem LMWMBP	362 ± 17	370 ± 16	347 ± 17	395 ± 17
Brain stem cholesterol	138 ± 4	133 ± 4	138 ± 4	136 ± 4
Brain stem glucocerebroside 1[d]	14.1 ± 0.9	15.2 ± 0.9	17.0 ± 0.9	15.8 ± 0.9
Brain stem glucocerebroside 2	38 ± 2	39 ± 2	40 ± 2	41 ± 2
Brain stem phosphatidylethanolamine	45 ± 2	46 ± 2	46 ± 2	45 ± 2
Brain stem phosphatidylcholine	89 ± 3	88 ± 3	92 ± 3	88 ± 3
Brain stem sphingomyelin	83 ± 3	85 ± 2	87 ± 3	86 ± 3
Cerebellum HMWMBP	189 ± 7	181 ± 7	189 ± 7	186 ± 7
Cerebellum LMWMBP	202 ± 12	201 ± 11	203 ± 11	210 ± 11
Cerebellum cholesterol	96 ± 3	92 ± 3	94 ± 3	95 ± 3
Cerebellum glucocerebroside 1	7.7 ± 0.8	8.6 ± 0.7	9.4 ± 0.7	7.6 ± 0.8
Cerebellum glucocerebroside 2	17 ± 1	18 ± 1	18 ± 1	18 ± 1
Cerebellum phosphatidylethanolamine	29 ± 2	30 ± 1	29 ± 1	28 ± 2
Cerebellum phosphatidylcholine	64 ± 3	61 ± 3	63 ± 3	62 ± 3
Cerebellum sphingomyelin	52 ± 3	52 ± 2	53 ± 3	53 ± 3
Spinal cord HMWMBP[b]	301 ± 8	272 ± 8	283 ± 7	292 ± 8
Spinal cord LMWMBP	308 ± 15	299 ± 14	299 ± 14	315 ± 14
Spinal cord cholesterol	137 ± 4	132 ± 3	133 ± 3	132 ± 4
Spinal cord glucocerebroside 1	12.9 ± 0.9	13.7 ± 0.8	14.9 ± 0.8	13.8 ± 0.9
Spinal cord	36 ± 2	35 ± 2	36 ± 2	37 ± 2

glucocerebroside 2				
Spinal cord phosphatidylethanolamine	45 ± 2	46 ± 2	46 ± 2	45 ± 2
Spinal cord phosphatidylcholine	83 ± 3	80 ± 3	82 ± 3	80 ± 3
Spinal cord sphingomyelin	92 ± 3	93 ± 3	94 ± 3	92 ± 3

[a] Main effect of Gromega treatment (P<0.05)
[b] Gromega x Zinc interaction (P<0.01)
[c] Gromega x Zinc interaction (P<0.05)
[d] Main effect of Zinc treatment (P<0.05)

Analysis of stomach contents, organ weights, and immunocrit values after adjusting for birth weights is summarized in Table 2. The Gromega by Zinc treatment interaction observed for unadjusted cerebellum weight became more statistically significant after adjustment with birth weight (P=0.01), but relationships between the means did not change. The effect of Gromega treatment on heart weight detected for unadjusted means disappeared after adjustment for birth weight, suggesting that this effect was due to an overall effect of Gromega treatment on size of the piglet and was not a specific effect of Gromega treatment on heart development. No other significant effects were obtained after adjustment for birth weight.

Table 2. Least squares means (± SEM) for stomach contents, organ weights, and immunocrit values after adjustment for birth weights.

Trait	Control	Gromega	Zinc	G + Z
Stomach contents, g	33 ± 2	30 ± 2	32 ± 2	31 ± 2
Brain weight, g	30.9 ± 0.3	30.1 ± 0.3	30.5 ± 0.3	30.4 ± 0.3
Brain stem weight, g	1.44 ± 0.02	1.38 ± 0.02	1.38 ± 0.02	1.38 ± 0.02
Cerebellum weight, g[a]	3.22 ± 0.04	3.01 ± 0.04	3.13 ± 0.04	3.13 ± 0.04
Heart weight, g	9.1 ± 0.1	9.2 ± 0.1	9.2 ± 0.1	8.9 ± 0.1
Immunocrit	0.127 ± 0.006	0.116 ± 0.006	0.123 ± 0.006	0.117 ± 0.006

[a] Gromega x Zinc interaction (P<0.01).

Results for myelin protein and lipid measurements after adjustment for myelin cholesterol measurements are presented in Table 3. The Gromega by zinc interaction that was significant for brain stem high molecular weight myelin basic protein, the zinc effect for brain stem glucocerebroside 1, and the Gromega by zinc interaction for spinal cord high molecular weight myelin basic protein detected using unadjusted data remained significant after adjustment for myelin cholesterol. In addition, there was a significant Gromega by zinc interaction detected for cerebellum glucocerebroside 1 (P=0.01) after adjustment for cerebellum myelin cholesterol. All other myelin traits remained nonsignificant.

Table 3. Least squares means (± SEM) for myelin measurements in the brain stem, cerebellum and spinal cord after adjustment for myelin cholesterol in each tissue.

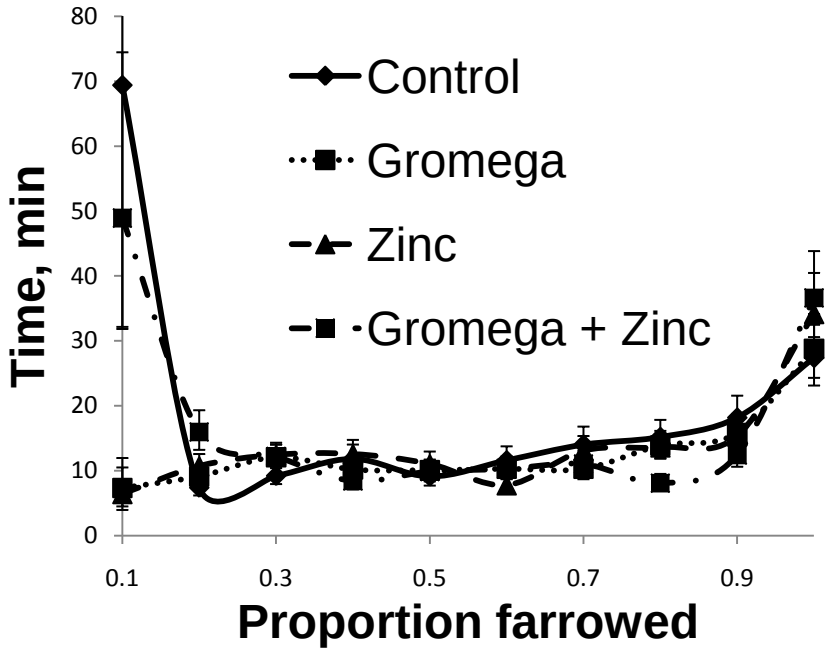
Trait	Control	Gromega	Zinc	G + Z
Brain stem HMWMBP[a]	338 ± 9	340 ± 9	323 ± 9	362 ± 9
Brain stem LMWMBP	360 ± 17	372 ± 16	345 ± 16	395 ± 17
Brain stem glucocerebroside 1[b]	13.8 ± 0.8	15.5 ± 0.8	16.7 ± 0.8	15.8 ± 0.8
Brain stem glucocerebroside 2	37 ± 1	40 ± 1	40 ± 1	41 ± 1
Brain stem phosphatidylethanolamine	45 ± 2	46 ± 2	45 ± 2	45 ± 2
Brain stem phosphatidylcholine	88 ± 2	89 ± 2	91 ± 2	88 ± 2
Brain stem sphingomyelin	82 ± 2	86 ± 2	86 ± 2	86 ± 2
Cerebellum HMWMBP	189 ± 7	180 ± 7	189 ± 7	187 ± 7
Cerebellum LMWMBP	201 ± 12	202 ± 11	204 ± 11	210 ± 11
Cerebellum glucocerebroside 1[c]	7.4 ± 0.6	8.9 ± 0.5	8.8 ± 0.6	7.5 ± 0.6
Cerebellum glucocerebroside 2	16.1 ± 0.9	18.7 ± 0.9	17.8 ± 0.9	17.4 ± 0.9
Cerebellum phosphatidylethanolamine	29 ± 2	30 ± 1	29 ± 1	28 ± 1
Cerebellum phosphatidylcholine	62 ± 2	63 ± 2	64 ± 2	61 ± 2
Cerebellum sphingomyelin	50 ± 2	53 ± 2	54 ± 2	53 ± 2
Spinal cord HMWMBP[c]	299 ± 7	272 ± 7	283 ± 7	293 ± 7
Spinal cord LMWMBP	306 ± 14	300 ± 14	299 ± 14	316 ± 14
Spinal cord glucocerebroside 1	12.5 ± 0.8	13.8 ± 0.8	14.9 ± 0.8	13.9 ± 0.8
Spinal cord glucocerebroside 2	35 ± 1	36 ± 1	36 ± 1	37 ± 1
Spinal cord phosphatidylethanolamine	45 ± 2	46 ± 2	46 ± 2	45 ± 2
Spinal cord phosphatidylcholine	81 ± 2	81 ± 2	82 ± 2	80 ± 2
Spinal cord sphingomyelin	90 ± 2	94 ± 2	94 ± 2	93 ± 2

[a] Gromega x zinc interaction ($P<0.05$)
[b] Main effect of zinc treatment ($P<0.05$)
[c] Gromega x zinc interaction ($P=0.01$)

Correlation analysis was used to determine whether any of the myelin traits measured had significant associations with immunocrit, weight of stomach contents or preweaning mortality. Immunocrit was significantly correlated ($r = 0.47$, $P<0.01$) with stomach contents, supporting the concept that immunocrits reflect piglet nursing ability. None of the myelin traits measured was significantly correlated with piglet stomach contents. By contrast, measurements of myelin basic protein in all three brain regions were weakly but significantly correlated with immunocrit (r range 0.16 to 0.23, $P<0.05$), with brain stem high molecular weight myelin basic protein having the greatest correlation. Because myelin basic protein measurements between brain regions were correlated with each other (r range 0.46 to 0.83, $P<0.01$), multiple regression was used to determine whether myelin basic protein measurements in various tissues contributed independently to variation in immunocrit measurement. This analysis indicated that only brain stem high molecular weight myelin basic protein

contributed independently to variation in immunocrit, simultaneous fitting of myelin basic protein measures in other brain tissues with brain stem high molecular weight myelin basic protein were nonsignificant. It was our hope that measurements of brain myelination in various tissues of the smallest piglet in each litter would be predictive of preweaning mortality of the entire litter. However, no correlations between any myelin traits measured and litter preweaning mortality were observed. Results indicated a significant effect of Gromega supplementation on the number of stillborn piglets per litter (Table 1), suggesting that Gromega supplementation may alter the farrowing process. Comparison of birth intervals between treatment groups did not indicate a significant effect of the Gromega treatment (Figure 2). There was a significant overall Gromega by zinc interaction and a significant Gromega by zinc by proportion of the litter farrowed interaction. However, examination of Figure 2 clearly indicates that the 0.1 proportion of the litter farrowed category in this experiment is not well estimated. Reanalysis of the data after omitting this category indicated only a zinc by proportion of the litter farrowed interaction, indicating that the previous significant results were the result of the poorly estimated 0.1 proportion of the litter category. The remaining zinc by proportion of the litter farrowed interaction appeared to be due to an increase in the birth interval of the last piglet in the litter for zinc treated gilts.

Figure 2: Least squares means (± SEM) for piglet birth intervals plotted against the proportion of the litter farrowed.



Discussion

In this experiment, we developed and validated a simple, rapid, inexpensive method for analysis of piglet serum IgG, the “immunocrit”, as a measure of the ability of newborn piglets to nurse successfully. Immunocrit measurements were significantly correlated with piglet stomach contents when piglets were sacrificed on day 1, supporting that the immunocrit reflects nursing ability of the piglet. Results indicated weak but significant correlations between immunocrit measurements and myelin basic protein measurements, but not lipid measurements, in the brain stem, cerebellum and spinal cord of the smallest piglets in litters. High molecular weight myelin basic protein in the brain stem had the greatest correlation. We also demonstrated that combined Gromega and zinc

supplementation of gilts from day 80 of gestation to farrowing increased high molecular weight myelin basic protein in the brain stem, although neither the combined treatment, nor either supplement alone, had any effect on immunocrit values, stomach content weights, or preweaning mortality. Gromega supplementation increased birth weights of the smallest piglets and the number of stillborn piglets, but did not affect the number of piglets born alive. The increase in the number of stillborn piglets was not explained by changes in piglet birth intervals after Gromega supplementation, however, zinc supplementation had the very curious effect of prolonging the birth interval of the last piglet in the litter. Despite the lack of effects of treatments on preweaning survival, these data suggest that brain myelination partially contributes to neonatal piglet nursing ability and that further experiments to improve brain myelination in small piglets may be useful in improving piglet preweaning survival.

Our results indicate that the immunocrit is as valid a measure of piglet serum IgG as the previous assay of Yaguchi et al. (1980). The two assays are both similar in that they are based on precipitation of IgG with ammonium sulfate. However, assessing precipitation using absorbance measurements is problematic because most samples have some endogenous absorbing material, primarily as the result of serum chylomicrons found in the blood after the piglet nurses. This necessitated performing an analysis of background absorbance in the Yaguchi et al. (1980) method, doubling the expense and time required to perform this assay. Our experiments indicate that this endogenous absorbance rises to the top of the microcapillary tube during the immunocrit assay, and therefore does not contribute to immunocrit measurement, such that analysis of background is not necessary. The immunocrit is also faster and requires less reagent than the procedure of Yaguchi et al. (1980). The immunocrit is predictive of piglet stomach contents ($r = 0.47$) despite no attempt to sacrifice the piglet at specific times after nursing. Thus, the immunocrit is predictive of nursing ability. We have also subsequently shown that the immunocrit assay performed on day 1 serum is predictive of preweaning mortality independent of the effect of piglet birth weights on preweaning mortality (Vallet, unpublished observations), which agrees with previous results (Kaguchi et al., 1980). The rapidity, ease and low expense of this assay, combined with the fact that it is predictive of preweaning mortality, suggests that this assay could be extremely useful in developing strategies to improve preweaning survival of piglets.

One of the objectives of this experiment was to demonstrate whether measurements of brain myelination were associated with measurements of piglet nursing ability or preweaning survival. In this experiment, the best demonstration that brain myelination is associated with piglet nursing ability is the weak but significant correlation between piglet brain stem high molecular weight myelin basic protein and piglet immunocrit values. However, no relationships were demonstrated between any of the treatments applied or traits measured and preweaning mortality. The experiment was designed to measure myelination of brain regions and its relationship with other traits. Assessment of brain myelination required sacrificing the smallest piglet in the litter, meaning that these piglets could not subsequently contribute to measurements of preweaning loss. This is reflected in the range of preweaning mortality of 7 to 9% measured in this experiment compared to the normal average in our herd of 12-13% (Vallet, unpublished). The lower values reflect the removal of the smallest, and therefore most at risk (Tuscherer, 2000), piglets from the analysis of preweaning mortality. This alone may explain the lack of effect of treatments on preweaning mortality. The other difficulty is that most of the measurements made in this experiment were done on the smallest piglet, with the hope that this would predict traits of the other piglets in the litter. Our best correlation between brain stem high molecular weight myelin basic protein and immunocrit measurements on the same piglet was only about 0.2, thus correlations on traits measured between piglets are likely to be even less, and it is not surprising that they were not detected.

Turning to the effects of Gromega or zinc supplementation on myelination, few were observed. The most significant effect occurred for high molecular weight myelin basic protein in the brain stem, which was previously demonstrated to be most affected by low birth weight (Vallet et al., 2010). A

similar but nonsignificant trend was observed for low molecular weight myelin basic protein in this brain region. A few other interaction effects between treatments were observed for protein and lipid myelination measurements in other brain regions but the nature of these interactions are difficult to interpret. It was our hypothesis that Gromega treatment would influence myelin lipid (Berkow and Campagnoni, 1983; Salvati et al., 1996; Yehuda et al., 2005; Boiko and Winckler, 2006), but this is not supported by the results. Myelin basic protein in combination with zinc is known to be involved in the compaction of myelin membranes around nerve axons (Kursula et al., 2008), a process essential to myelination. The roles of high and low molecular weight isoforms of myelin basic protein in this process are not known. Despite the lack of dietary supplement effects on immunocrit values or preweaning mortality, the increase in high molecular weight myelin basic protein in the brain stem combined with the association between brain stem high molecular weight myelin basic protein and immunocrit values suggest that combined Gromega and zinc supplementation may be beneficial to the newborn piglet. Further experimentation with these supplements is necessary to fully evaluate the combination of Gromega and zinc supplementation on preweaning mortality.

Other observed effects of Gromega and/or zinc supplementation on traits measured were surprising. Gromega supplementation increased the heart and birth weight of the smallest piglet of the litter, but did not affect average birth weight. Further analysis indicated that the observed differences in heart weight were the result of differences in birth weight. Gromega supplementation increased the number of stillborn piglets but did not affect the number of piglets born alive, weaned or preweaning mortality. Examination of birth intervals in Gromega-treated gilts provided no explanation for the increase in stillborn piglets, no significant differences in birth intervals in Gromega-treated gilts were observed. In contrast, a significant zinc by proportion of the litter farrowed interaction was observed, which appeared to be due to an effect of zinc supplementation on the birth interval of the last piglet farrowed in each litter. Birth of the last piglet in the litter is often significantly delayed compared to the rest of the litter (Vallet et al., 2010) and the mechanism responsible for this delay is not clear. We have hypothesized that the increased length of the last birth interval is due to the lack of ability of a single remaining piglet to support the birth process, and have suggested that this could be due to a lack of sufficient synthesis of prostaglandin E by the single remaining unborn piglet placenta. Zinc is a component of copper/zinc superoxide dismutase, which is a scavenger of superoxide radicals (Abreu and Cabelli, 2010). Kelner and Uglik (1995) reported that copper/zinc superoxide dismutase interferes with the induction of prostaglandin E in fibroblasts, providing a possible mechanism for the effect of zinc supplementation on the birth interval of the last piglet. Whether this effect of zinc supplementation is mediated by copper/zinc superoxide dismutase effects on the synthesis of PGE by the placenta requires further research.

In summary, we have developed a simple, rapid, inexpensive assay to assess passive transfer of IgG from dam to piglet, which can be used as an indicator of neonatal piglet nursing ability. Measurement of myelin basic protein and myelin lipid in the brain stem, cerebellum and spinal cord of the smallest piglet in each litter indicated a significant but low correlation between brain stem high molecular weight myelin basic protein and immunocrit values. The combined dietary Gromega and Zinc supplementation of gilts from day 80 of gestation onward increased brain stem high molecular weight myelin basic protein, but did not affect weight of the stomach contents or immunocrit measurements in the smallest piglets in the litter, nor did the combined supplementation affect overall preweaning mortality. The immunocrit assay will be useful in assessing newborn piglet nursing ability and further research is needed to determine whether combined Gromega and zinc supplementation of gilt diets will improve preweaning mortality.

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References

Abreu IA, Cabelli DE. 2010. Superoxide dismutases-a review of the metal-associated mechanistic variations. Biochim. Biophys. Acta 1804: 263-274.
Arango J, Misztal I, Tsuruta S, Culbertson M, Holl JW, Herring W. 2006. Genetic study of individual preweaning mortality and birth weight in Large White piglets using threshold-linear models. Livestock Sci. 101: 208-218.
Bereskin B, Shelby CE, Cox DF. 1973. Some factors affecting pig survival. J. Anim. Sci. 36: 821-827.
Berkow SE, Campagnoni AT. 1983. Essential fatty acid deficiency: effects of cross-fostering mice at birth on myelin levels and composition. J. Nutr. 113: 582-591.
Boiko T., Winckler B. 2006. Myelin under construction-teamwork required. J. Cell Biol. 172: 799-801.
Buhi WL, Vallet JL, Bazer FW. 1989. De novo synthesis and release of polypeptides from cyclic and early pregnant porcine oviductal tissue in explants culture. J. Exp. Zool. 252: 79-88.
Deber CM, Reynolds SJ. 1991. Central nervous system myelin: structure, function, and pathology. Clin. Biochem. 24: 113-134.
Dickerson, JWT, Merat A, Widdowson EM 1971. Intra-uterine growth retardation in the pig. III. The chemical structure of the brain. Biol. Neonate 19: 354-362.
Fahmy MH, Bernard C. 1971. Causes of mortality in Yorkshire pigs from birth to 20 weeks of age. Can. J. Anim. Sci. 51: 351-359.
Fahmy MH, Holtmann WB, MacIntyre M, Moxley JE. 1978. Evaluation of piglet mortality in 28 two-breed crosses among eight breeds of pig. Anim. Prod. 26: 277-285.
Fields, RD, 2008. White matter matters. Scientific American 298: 42-49.
Ghosh SK, Rawal N, Syed SK, Paik WK, Kim S. 1991. Enzymic methylation of myelin basic protein in myelin. Biochem. J. 275: 381-387.
Horrocks LA. 1967. Composition of myelin from peripheral and central nervous systems of the squirrel monkey. J. Lipid Res. 8: 569-576.
Horrocks LA. 1968. The alk-1-enyl group content of mammalian myelin phosphoglycerides by quantitative two-dimensional thin-layer chromatography. J. Lipid Res. 9: 469-472.
Kelner MJ, Uglik SF. 1995. Superoxide dismutase abolishes the platelet-derived growth factor-induced release of prostaglandin E_2 by blocking induction of nitric oxide synthase: Role of superoxide. Arch. Biochem. Biophys. 322: 31-38.
Kursula P. 2008. Structural properties of proteins specific to the myelin sheath. Amino Acids 34: 175-185.
Le Dividich J, Herpin P, Rosario-Ludovino RM. 1994.Utilization of colostral energy by the newborn piglets. J. Anim. Sci 72: 2082-2089.
Noblet J, Dourmad JY, Etienne M, Le Dividich J. 1997. Energy metabolism in sows and newborn pigs. J. Anim. Sci. 2708-2714.
Pond WG, Boleman SL, Fiorotto ML, Ho H, Knabe DA, Mersmann HJ, Savell JW, Su DR. 2000. Perinatal ontogeny of brain growth in the domestic pig. Proc. Soc. Exp. Biol. Med. 223:102-108.
Salvati S, Sanchez M, Campeggi LM, Suchanek G, Breitschop H, Lassmann H. 1996. Accelerated myelogenesis by dietary lipids in rat brain. J. Neurochem. 67: 1744-1750.
Tuchscherer M, Puppe B, Tuchscherer A, Tiemann U. 2000. Early identification of neonates at risk: traits of newborn piglets with respect to survival. Theriogenology 54: 371-388.
Vallet JL, Miles JR. 2010. Myelination is decreased in the brain stem of small piglets compared to larger littermates during late gestation. J. Anim. Sci. 88, E-Suppl. 3: Abstr. 249.
Vallet JL, Miles JR, Brown-Brandl TM, Nienaber JA. 2010. Proportion of the litter farrowed, litter size, and progesterone and estradiol effects on piglet birth intervals and stillbirths. Anim. Reprod. Sci. 119: 68-75.
Van der Lende T. 2001. Prenatal development as a predisposing factor for perinatal losses in pigs. Reprod. Suppl. 58: 247-261.

Yaguchi H, Murata H, Kagota K, Namioka S. 1980. Studies on the relationship between the serum gamma globulin levels of neonatal piglets and their mortality during the first two months of life: an evaluation for the ammonium suphate reaction. Br. Vet. J. 136: 63-70.
Yehuda S, Rabinovitz S, Mostofsky DI. 2005. Essential fatty acids and the brain: From infancy to aging. Neurobiology of Aging 26S:S98-S102.