

Effect of mixing piglets affected by *Escherichia coli* diarrhea on growth and welfare responses

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Summary

Objective: To evaluate the effects of mixing piglets prior to weaning on growth and behavioral and physiological stress markers during the preweaning and postweaning periods in the face of an outbreak of *Escherichia coli* diarrhea.

Materials and methods: Twenty-four sows and their litters from two adjacent rooms (A and B, 12 litters per room), and six focal piglets (three males and three females) from each litter were included. In Room B, *E coli* diarrhea occurred Day 12 after birth. On Day 18, the partitions between pairs of neighboring pens were

removed for 12 litters, allowing piglets access to two adjoining crate areas (forming six mixed litters). Pigs were weaned on Day 28. Growth performance, behavioral stress markers, and physiological stress markers (neutrophil:lymphocyte ratio and acute phase proteins [APPs]) were measured from birth to Day 58.

Results: When colibacillosis occurred in Room B, there was a detrimental effect on growth performance, especially highlighted when piglets were mixed prior to weaning. Concentrations of APPs 2 days after weaning were higher in Room B. Mixed piglets spent more time fighting immediately after

mixing; however, after weaning, mixed piglets spent less time fighting than the controls.

Implications: Mixing piglets affected by *E coli* diarrhea may have a detrimental effect on growth rate. Animals with colibacillosis may have higher APP concentrations. After weaning, pigs mixed pre-weaning spend less time fighting than controls, which might help them cope with the stress of weaning.

Keywords: swine, mixing, diarrhea, growth, stress markers

Received: November 2, 2011

Accepted: April 30, 2012

Resumen - Efecto de la mezcla de lechones afectados con diarrea por *Escherichia coli* en respuesta al crecimiento y bienestar

Objetivo: Evaluar los efectos de la mezcla de lechones antes del destete en los marcadores de crecimiento y estrés fisiológico y de conducta durante los periodos de predestete y post destete frente a un brote de diarrea por *Escherichia coli*.

Materiales y métodos: Se incluyeron veinticuatro hembras y sus camadas, de dos cuartos contiguos (A y B, 12 camadas por cuarto), y seis lechones focales (tres machos y tres hembras) de cada camada. En el Cuarto B, la diarrea de *E coli* ocurrió en el Día 12 después del parto. En el Día 18, las divisiones entre los pares de corrales contiguos se

quitaron para 12 camadas, permitiendo a los lechones el acceso a dos áreas de corrales contiguos (formando seis camadas mixtas). Los cerdos se destetaron en el Día 28. El desempeño del crecimiento, los marcadores de estrés conductual, y los marcadores de estrés fisiológico (relación neutrófilo:linfocito y proteínas de fase aguda) se midieron desde el parto al Día 58.

Resultados: Cuando ocurrió la colibacilosis en el Cuarto B, hubo un efecto detrimental en el desempeño del crecimiento, especialmente marcado cuando los lechones se mezclaron antes del destete. Las concentraciones de las proteínas de fase aguda 2 días después del destete fueron más altas en el Cuarto B. Los lechones mezclados pasaron más tiempo

peleando inmediatamente después de mezclarse; sin embargo, después del destete, los lechones mezclados pasaron menos tiempo peleando que los controles.

Implicaciones: Mezclar lechones afectados por la diarrea de *E coli* puede tener un efecto detrimental en el índice de crecimiento. Los animales con colibacilosis pueden tener concentraciones de las proteínas de fase aguda más altas. Después del destete, los cerdos mezclados predestete, pasan menos tiempo peleando que los controles, lo que puede ayudarles a sobrellevar el estrés del destete.

Résumé - Effet du mélange de porcelets souffrant de diarrhée à *Escherichia coli* sur leur croissance et leur bien-être

Objectif: Évaluer les effets de mélanger des porcelets préalablement au sevrage sur la croissance et des marqueurs de stress physiologiques et comportementaux durant les périodes pré- et post-sevrage lors d'un épisode de diarrhée à *Escherichia coli*.

Matériels et méthodes: Vingt-quatre truies et leur portée logées dans deux chambres adjacentes (A et B, 12 portées par chambre) et six porcelets (trois mâles et trois femelles) de chaque portée ont été inclus dans l'étude. Dans la Chambre B, la diarrhée à *E coli* est

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This article is available online at <http://www.aasv.org/shap.html>.

Quiñonero J, Ramis G, Lopes E, et al. Effect of mixing piglets affected by *Escherichia coli* diarrhea on growth and welfare responses. *J Swine Health Prod.* 2012;20(5):216–222.

survenue au Jour 12 après la naissance. Au Jour 18, les séparations entre les paires de parcs voisins ont été retirées pour 12 portées, permettant aux porcelets d'avoir accès à deux zones de cage adjacentes (formant ainsi six portées mélangées). Les porcs ont été sevrés au Jour 28. Les performances de croissance, des marqueurs de stress comportementaux, et des marqueurs de stress physiologiques (ratio neutrophile:lymphocyte et protéines de phase aigüe [APP]) ont été mesurés de la naissance au Jour 58.

Résultats: Lorsque la colibacillose est survenue dans la Chambre B, il y eut un effet délétère sur les performances de croissance, mis en évidence surtout lorsque les porcelets ont été mélangés préalablement au sevrage. Les concentrations d'APP 2 jours après le sevrage étaient plus élevées dans la Chambre B. Les porcelets mélangés ont passé plus de temps à se battre immédiatement après avoir été mélangés; toutefois, après le sevrage, les porcelets mélangés ont passé moins de temps à se battre que les témoins.

Implications: Mélanger des porcelets souffrant de diarrhée à *E coli* peut avoir des effets délétères sur le taux de croissance. Des animaux avec la colibacillose peuvent avoir des concentrations plus élevées d'APP. Après le sevrage, les porcs mélangés pré-sevrage passent moins de temps à se battre que les témoins, ce qui pourrait les aider à passer au travers le stress du sevrage.

Maintaining optimum animal welfare and health is of primary importance in pig production. Factors related to animal health, security of the feed, environmental criteria, and animal welfare concerns are increasingly taken into consideration by consumers, and therefore are included in quality production criteria. Generally, the “five freedoms” rule proposed by the UK Farm Animal Welfare Council¹ have been well accepted: animals must be free from hunger, thirst, and malnutrition; from discomfort; from pain, injury, and disease; and from fear and distress; and must be free to express normal patterns of behavior.

Pigs' reactions to stressful situations can affect behavior, the autonomic nervous system, or the neuroendocrine or immune systems.² In modern production systems, farm livestock experience a variety of stressors that can modify normal behavior and growth, with resultant poor performance.³⁻⁶

Pigs from different groups are commonly mixed during gestation, at weaning, and at the beginning of the growing-finishing period. They must cope with environmental changes, yet it is well known that pigs exhibit novel-induced anxiety under these circumstances.³ At the same time, pigs must cope with social challenges that may cause injuries and physiological reactions to acute stress. Fighting among newly mixed pigs (ie, during the first 24 hours after mixing) is part of the process necessary to establish a dominance order.⁴ In addition, Weary et al⁵ have reported detrimental effects on growth rate in the period between mixing of suckling pigs and weaning.⁵ In another experiment,⁶ behavioral and physiological abnormalities and depressed growth were induced by repeated regrouping and relocation of pigs. However, to our knowledge, no published studies have addressed the effect on physiological stress markers when piglets are mixed during an outbreak of *E coli* diarrhea.

Weaning is an especially stressful situation because piglets have to face a new environment, unfamiliar piglets, and separation from their dams. They no longer receive passive protection afforded by antibodies in milk, and at the same time the stressful situation causes depression of the immune system. All of these factors can be highly stressful, compromising animal welfare, and, in the end, can result in an increased incidence of disease.^{7,8} Litters of piglets that are mixed before weaning and are accustomed to new partners at an early age may cope better with the stress of weaning. However, infectious diarrhea, which is common during the lactation period, may become widespread when litters are mixed.

The aim of this study was to evaluate the effects of mixing suckling piglets (litters sharing dams and floor space) on growth and behavioral and physiological markers of stress during the preweaning and postweaning periods when diarrhea appeared at early age.

Materials and methods

The Bioethical Committee on Animal Experimentation of the University of Murcia approved this trial. The animals were reared according to European Union (EU) and Spanish regulations regarding welfare.^{9,10}

Animals and housing systems

This experiment was conducted on 144 Large White × (Large White × Landrace) piglets in a large commercial piggery in the

southeast of Spain, beginning in October 2007 and concluding in December 2007. Animals from 24 litters from two adjacent rooms (A and B) were used, with 12 litters per room. Piglets were individually identified by ear tags. A random number generator was used to select six focus piglets (three males and three females) from each litter for data collection. Each room was divided into two sides by a central corridor, with six farrowing pens (2.1 m × 2 m) per side. Each farrowing pen was partially slatted (slats at the back) and was equipped with a crate for the dam with a trough and a drinker at the front. For the piglets, a drinker was placed at the back of the slatted area and a trough for the starter diet was placed at the front of the unslatted area, which was heated by a radiant floor heating system with infrared light support. Sawdust was used on the floor of this area. Pens were illuminated by overhead fluorescent lighting (30 lux). Pens were separated by metal dividers so that minimal contact was possible between neighboring pigs. Routine tail docking, teeth clipping, intramuscular iron dextran injections, and castration of males were performed within 24 hours of birth. In the first 5 days of life, pigs were cross-fostered to create litters of approximately 10 to 12 piglets. Sows were fed twice a day and a starter concentrate was provided to the piglets beginning at 14 days of age.

Day 0 was defined as the day of birth and pigs were weaned on Day 28 (at 4 weeks of age as required by EU legislation⁸). Ten days before weaning (Day 18), dividers separating pairs of neighboring pens were removed on one side of each room (12 litters total), thus allowing two litters to mix (Mixed group; six focus piglets per litter, 72 focus piglets total). On the other side of each room, litters were not mixed and the piglets remained in the same pen with their siblings until weaning (Control group; 12 litters total, six focus piglets per litter, 72 focus piglets total).

Weaned pigs were housed in fully slatted pens until they reached a weight of 20 kg (nursery period). Nursery pen size was 3 m × 3 m, with 35 to 40 pigs per pen. In each pen, piglets originated from three or four litters from the same room, with entire litters penned together (ie, littermates were not separated). Feed and water were available ad libitum from one feeder and one drinker per pen. A pelleted standard diet for growing pigs was provided. Pens were illuminated by overhead fluorescent lighting (30 lux).

Health status

The sows were carriers of porcine reproductive and respiratory syndrome virus (PRRSV), *Mycoplasma hyopneumoniae*, *Lawsonia intracellularis*, and porcine circovirus type 2 (PCV2) as diagnosed by serology and polymerase chain reaction (PCR), but were free of Aujeszky's disease virus, mange, and swine dysentery. Testing was performed as part of the herd's routine health surveillance program. Clinical signs of PRRSV-, PCV2-, or *M. hyopneumoniae*-related diseases were not identified during the 12 months prior to the experiment. Sporadic outbreaks of *E. coli* diarrhea had occurred in suckling and nursery pigs, diagnosed by histopathology observations, conventional bacterial isolation procedures, and PCR in the Histopathology Laboratory and Genomic Laboratory of the University of Murcia. Animals were not medicated on a constant basis or vaccinated during the experimental procedure in order to avoid altering the acute phase protein (APP) assessments.

Growth rate

During the experiment, all pigs were weighed individually at birth and after that on a weekly basis until the end of the experiment (at 58 ± 2 days of age) on an electronic scale accurate to 50 g. Average daily gain (ADG; g per day) was determined for the period between Day 0 (the day the pigs entered the experiment) and Day 58 (1 month after weaning).

Neutrophil:lymphocyte ratio

Blood samples were collected from each focus pig 2 days after mixing (Day 20), 2 days after weaning (Day 30), and at the end of the experiment (Day 58). On Days 20 and 58, capillary samples were collected by pricking the skin of the ear with a needle; the resulting drop of blood was placed directly on a microscope slide. However, the Day 30 sample was collected by jugular venipuncture into an EDTA tube. A small amount of this sample was immediately used to determine the neutrophil:lymphocyte (N:L) ratio. A smear was made and stained using the May-Grünwald-Giemsa method.¹¹ Neutrophils and lymphocytes (approximately 100 cells total) were counted by microscopic examination. The N:L ratio was determined by dividing the number of neutrophils by the number of lymphocytes.

Acute phase proteins

The remainder of the Day 30 blood sample

was centrifuged and the plasma was used to determine the serum concentrations of three APPs: C-reactive protein (CRP), serum amyloid A (SAA), and haptoglobin (Hp). The concentration of serum Hp was determined by the hemoglobin binding method¹² on a MIRA biochemical analyzer (Roche Diagnostics, Mannheim, Germany). The inter-assay coefficient of variance (CV) was 5.3%, the intra-assay CV was 1.5%, and the limit of detection was 0.02 mg per mL. Serum amyloid A was assayed using the Phase SAA Assay Kit (Tridelta Development Ltd, Bray Co, Wicklow, Ireland), according to the manufacturer's instructions. The inter-assay CV was < 8%, the intra-assay CV was < 12%, and the limit of detection was 5 ng per mL. Serum concentration of CRP was determined by enzyme-linked immunosorbent assay (ELISA) using the Phase Porcine CRP (Tridelta Development Ltd) according to the manufacturer's instructions. The inter-assay CV was 6.1%, the intra-assay CV was 3.1%, and the limit of detection was 0.01 µg per mL. All three APP assays were performed in duplicate.

Behavior

Experimental pens were videotaped for 90 minutes the day after commingling (Day 19), and the day after weaning (Day 29) by scan sampling with an interval between scans of

1 minute, according to Coutellier et al.⁶ Pens were videotaped using six cameras (SSC-G923 1/3; Sony Corporation, Osaka, Japan), one camera per litter. One side of a room was recorded, then the cameras were moved to the other side and so on until both sides of Rooms A and B were recorded. The cameras were connected to two time-lapse video recorders (TL700) via two switcher systems (ATV DPX; Panasonic Corp, Osaka, Japan). Three focus males per litter were individually identified using different colors of stock marker spray painted on their backs. Behavior data were collated according to a predetermined ethogram (Table 1) based on the method of Day et al,¹³ and the frequency at which each category of the ethogram occurred was expressed as a percentage of the total number of observations.

Statistical analyses

A linear mixed effects model was fitted to the data (body weight, ADG, N:L ratio, APPs, and behavior), using the nlme package (Linear and Nonlinear Mixed Effects Models [<http://cran.r-project.org/web/packages/nlme//index.html>]) of the R statistical computing environment (R Foundation for Statistical Computing, Vienna, Austria, 2009), where mixing, room, and gender were considered fixed effects, while litter was included as a random effect. Inter-

Table 1: Experimental ethogram* used to analyze behavior of piglets the day after mixing (19 days of age) and the day after weaning (29 days of age)†

Behavior	Description
Alert	Motionless (eyes open, ears erect)
Inactive	Motionless (eyes closed)
Nosing substrate‡	Nose close to substrate
Rooting substrate‡	Displacing substrate with circular movements of the snout
Feeding	Eating from feeder
Drinking	Drinking from water drinker
Fighting	Interacting aggressively with another pig
Other	Other unlisted activity

* Ethogram adapted from Day et al (2002).¹³

† In Rooms A and B, individually identified male piglets were observed by videotape recording for 90-minute periods with an interval of 1 minute between scans. Pairs of neighboring litters on one side of the room were commingled beginning at 18 days of age (Mixed group; six mixed litters; n = 36 pigs). Three male piglets per litter were selected for observation from each of the Mixed group litters in each room, and three male piglets were selected for observation from each litter on the second side of each room (Control group; 12 unmixed litters; n = 36 pigs).

‡ Substrate refers to other pigs, penning, feeder, and drinker for nosing, and to sawdust, manure, or spilled feed for rooting.

action between mixing and room effects was included if it was significant ($P < .05$). Least squares differences (LSD) intervals have been represented for means comparison. Gender effect was not included in behavior analysis as only males were used.

Results

Growth performance and health assessment

A significant interaction ($P < .05$) was found between mixing and room effects for weaning weight and for ADG from birth to weaning (ADG_{0-W}) (Table 2). As a consequence, there was no significant effect of mixing piglets on growth performance in Room A; however, in Room B, weaning weight and ADG_{0-W} were significantly lower in Mixed piglets. In addition, weaning weight was lower in Controls in Room B than in Room A, and the difference between rooms was greater ($P < .05$) in the Mixed pigs. Birth weight did not differ between Room A and B, but by Day 58, Room B pigs weighed approximately 3 kg less than Room A pigs. There was no gender effect on growth performance.

During the experiment, diarrhea occurred in Room B piglets at an early age. Colibacillosis was diagnosed at Day 12 on the basis of clinical, histopathological, and microbiological assessments. Drinking water was medicated with colistin at a dosage of 5 mg per kg weight, and severely affected piglets were treated parenterally with enrofloxacin, IM, at 2.4 mg per kg weight. The initial prevalence was 25% of animals and mortality during the outbreak was 0.1%. As expected, diarrhea became more widespread when the animals were mixed, increasing the prevalence to 40% of piglets (Day 20). The outbreak was completely controlled 7 days after its maximal prevalence (Day 27).

Blood stress markers

The mean N:L ratio did not differ significantly before or after the piglets were mixed in litters and also did not differ in Rooms A and B. However, the ratio was 52% higher 2 days after weaning (Day 30) than on Day 20 (Table 3). The effect of weaning meant an increase in neutrophils and a decrease in lymphocytes and therefore a higher N:L

ratio. On Day 58, the N:L ratio did not differ from that on Day 20, two days after piglets were mixed.

The concentrations of the three studied APPs are shown in Table 3. There were significant differences ($P < .05$) between Room A and B for CRP and Hp concentrations, with values of CRP and Hp higher in Room B (21% and 40% of the overall mean values, respectively), but values did not differ between Mixed and Control pigs ($P > .05$). Mean concentration of SAA was higher in females than in males.

Behavior

Behavior results are presented in Table 4 (Day 19) and Table 5 (Day 29). When both tables were analyzed concurrently, we observed that pigs spent a large amount of the observation period lying down, and this was greater for piglets at Day 19 (Table 4) than for 29-day-old pigs (Table 5). The second behavior in which piglets spent a large percentage of time was nosing (penmates, parts of the pen dividers or floor, feeder, and drinker), especially after weaning. Also

Table 2: Least squares means and standard error of the means (SE) for weights and average daily gain of Mixed and Control pigs housed in Rooms A and B*

	Mixing effect		Room effect		Gender effect	
	Mixed	Control	A	B	Female	Male
	n = 72	n = 72	n = 72	n = 72	n = 72	n = 72
Body weight (SE) (kg)						
Birth	1.64 (0.32)	1.50 (0.35)	1.54 (0.30)	1.58 (0.30)	1.53 (0.32)	1.59 (0.36)
10 days	3.54 (0.76)	3.51 (0.66)	3.73 ^a (0.68)	3.30 ^b (0.74)	3.48 (0.71)	3.57 (0.71)
20 days	5.80 (0.14)	5.58 (0.88)	6.19 ^a (0.11)	4.94 ^b (128)	5.59 (0.12)	5.76 (0.11)
Weaning†	A 7.91 ^a (0.21)	7.51 ^a (0.16)	NA	NA	7.15 (0.15)	7.31 (0.12)
	B 5.23 ^b (0.13)	6.61 ^c (0.17)	NA	NA		
40 days	9.09 (0.17)	9.47 (0.18)	9.87 ^a (0.16)	8.48 ^b (0.19)	9.16 (0.18)	9.42 (0.16)
50 days	11.2 (0.23)	12.1 (0.25)	12.3 ^a (0.22)	10.8 ^b (0.28)	11.5 (0.25)	12.0 (0.24)
58 days (final)	13.6 (0.30)	14.5 (0.36)	15.6 ^a (0.32)	12.2 ^b (0.34)	14.0 (0.34)	14.2 (0.33)
Average daily gain (SE) (g/d)						
Birth-weaning	A 248 ^a (6.9)	240 ^a (5.1)	NA	NA	228 (5.1)	237 (4.3)
	B 148 ^b (6.3)	231 ^a (6.9)	NA	NA		
Weaning-final	211 (7.1)	221 (8.1)	224 (7.1)	204 (8.7)	215 (7.6)	220 (8.2)

* In Room A and Room B, three male and three female focus pigs were selected from each Mixed and Control litter (n = 72 pigs, three males and three females per litter, 12 litters per treatment). Focus pigs were weighed at birth and at 10, 20, 28 (weaning), 40, 50, and 58 days of age. Pigs were mixed as described in Table 1 and weaned at 28 days of age, and the experiment ended when they were 58 days of age.

† Significant interaction between mixing and room effects ($P < .05$; least squares differences).

^{abc} Means with different superscript letters within the same effect and parameter differ ($P < .05$; least squares differences)

NA = not applicable.

Table 3: Least squares (LS) means and standard error of the mean (SE) for blood stress markers (neutrophil:lymphocyte ratio and acute phase proteins) in piglets in litters either allowed to commingle with one neighboring litter at 18 days of age (Mixed) or not (Control)*

Parameters	LS means (SE)					
	Mixing effect		Room effect		Gender	
	Mixed	Control	A	B	Female	Male
	n = 72	n = 72	n = 72	n = 72	n = 72	n = 72
N:L20days	0.63 (0.01)	0.63 (0.01)	0.65 (0.01)	0.61 (0.01)	0.64 (0.01)	0.63 (0.01)
N:L30days	0.96 (0.02)	0.93 (0.01)	0.97 (0.02)	0.93 (0.02)	0.96 (0.02)	0.95 (0.02)
N:L58days	0.65 (0.01)	0.65 (0.01)	0.67 (0.01)	0.63 (0.01)	0.64 (0.01)	0.66 (0.01)
CRP (µg/mL)	18.12 (0.99)	15.51 (0.94)	15.67 ^a (0.80)	19.32 ^b (1.29)	17.51 (1.13)	15.89 (0.90)
SAA (µg/mL)	7.31 (1.19)	7.58 (1.37)	7.21 (1.10)	8.10 (1.68)	9.73 ^a (1.58)	5.24 ^b (0.98)
Hp (mg/mL)	1.38 (0.08)	1.34 (0.10)	1.20 ^a (0.06)	1.78 ^b (0.17)	1.44 (0.08)	1.28 (0.10)

* Mixing procedure and selection of focus pigs for blood sampling described in Tables 1 and 2, respectively. Blood samples were collected from focus pigs at 20 days of age (2 days after commingling), at 30 days of age (2 days post weaning), and at 58 days of age (the end of the experiment). Acute phase proteins were assayed only at 30 days of age.

^{ab} Means with different superscripts within the same effect and stress marker are significantly different ($P < .05$; least squares differences).

N:L = neutrophil:lymphocyte ratio; CRP = C-reactive protein; SAA = serum amyloid A; Hp = haptoglobin.

after weaning, pigs began rooting (sawdust, manure, or spilled feed). Pigs spent more time eating and less time fighting at Day 19 than at Day 29 when this comparison is made in the Controls.

The day after mixing (Day 19; Table 4), the Mixed piglets spent more time nosing and fighting, and consequently less time inactive ($P < .05$). The percentage of time spent eating or drinking did not differ between Mixed and Control pigs or between Room A and Room B pigs. On Day 19, the greatest difference between Mixed and Control groups was for fighting behavior, with Mixed piglets spending a greater percentage of time fighting. After weaning (Day 29; Table 5), the difference between Mixed and Control groups was more marked. However, Mixed piglets spent a greater percentage of time fighting after mixing than after weaning (8.60% versus 0.34%) and consequently, after weaning, Mixed pigs spent a smaller percentage of time fighting than did the Controls (0.34% versus 5.19%).

Behavior of pigs in Rooms A and B did not differ on Day 19, but after weaning, Room A pigs spent a greater percentage of time on investigatory behavior (nosing) than did Room B pigs, which spent more time inactive (Table 5).

Table 4: Least squares (LS) means and standard error (SE) of the mean for behavior (% time spent in an activity) in 19-day-old male piglets that had been either commingled with a neighboring litter at 18 days of age (Mixed) or not commingled (Control)*

Behavior	LS means (SE)(% of time)			
	Mixing effect		Room effect	
	Mixed	Control	Room A	Room B
	n = 36	n = 36	n = 36	n = 36
Inactive	69.2 ^a (0.72)	77.7 ^b (0.72)	73.1 (0.72)	73.8 (0.72)
Alert	2.2 (0.20)	1.7 (0.20)	1.6 (0.20)	2.2 (0.20)
Nosing	11.5 ^a (0.60)	8.6 ^b (0.60)	10.4 (0.60)	9.8 (0.60)
Fighting	8.6 ^a (0.46)	2.8 ^b (0.46)	5.9 (0.46)	5.6 (0.46)
Feeding	7.2 (0.61)	7.3 (0.61)	7.2 (0.61)	7.3 (0.61)
Drinking	0.6 (0.15)	0.6 (0.15)	0.7 (0.15)	0.6 (0.15)
Other	0.8 (0.26)	1.4 (0.26)	1.1 (0.26)	1.0 (0.26)

* Mixing procedure described in Table 1. The day after Mixed litters were commingled, scan samples of focus piglets (three males per litter; 36 piglets per room) were collected by videotaping for 90 minutes with an interval between scans of 1 minute. Time spent in a behavior is expressed as the LS mean of the percent of the total time piglets were videotaped. Ethogram categories (behaviors) are described in Table 1.

^{ab} Values with different superscript letters within an effect and ethogram category (behavior) differ ($P < .05$; least squares differences)

Table 5: Least squares (LS) means and standard error (SE) of the mean for behavior (% time spent in an activity) in 29-day-old male pigs that had been weaned at 28 days of age and either commingled with a neighboring litter at 18 days of age (Mixed) or not commingled (Control)*

Behavior	LS means (SE)(% of time)			
	Mixing effect		Room effect	
	Mixed	Control	Room A	Room B
	n = 36	n = 36	n = 36	n = 36
Inactive	61.5 ^a (1.41)	67.1 ^b (1.41)	61.5 ^a (1.68)	67.0 ^b (1.89)
Alert	3.8 ^a (0.30)	1.8 ^b (0.30)	3.1 (0.35)	2.6 (0.25)
Nosing	15.1 (1.01)	18.5 (1.01)	19.5 ^a (1.20)	14.0 ^b (0.85)
Fighting	0.3 ^a (0.59)	5.2 ^b (0.59)	2.4 (0.70)	3.2 (0.49)
Feeding	4.5 (0.43)	4.4 (0.43)	4.5 (0.51)	4.5 (0.36)
Drinking	0.8 (0.13)	0.7 (0.13)	0.8 (0.15)	0.7 (0.11)
Rooting	4.1 (0.84)	4.1 (0.84)	4.5 (0.99)	3.8 (0.71)
Other	6.5 (0.86)	1.7 (0.86)	3.9 (1.02)	4.3 (0.72)

* Piglets were weaned at 28 days of age (Day 28) and scan sampling of activities (described in Table 4) was performed the next day (Day 29). Ethogram categories (behaviors) are described in Table 1.

^{ab} Values with different superscript letters within the same effect and ethogram category (behavior) differ ($P < .05$; least squares differences).

Discussion

Several investigators^{5,14,15} have studied the effect of mixing piglets in the absence of a contagious disease, and these studies found that mixing piglets had no effect on growth performance. We found similar results when we analyzed Room A where infectious diarrhea did not occur. Parratt et al¹⁴ observed no effect on pre-weaning or postweaning growth rate when piglets were mixed 5 days before they were weaned at 28 days of age. However, in that study,¹⁴ piglets were mixed closer to the day of weaning than in the present study, and the authors pointed out that the interval between mixing and weaning may affect growth rate. When this interval is long, disruptions to suckling behavior may have detrimental effects on growth rate. However, in Weary et al,⁵ piglets were mixed at only 11 days of age and were weaned at 28 days of age. These authors observed that mixed piglets tended to gain less weight before weaning, but weaning was less stressful for them and they gained more weight post weaning. In the end, growth for the entire period was similar to that of the control group. Kanaan et al¹⁵ mixed piglets at 13 days of age, and reported that this had no effect on growth either for the whole experiment or at any time point (from birth to 18 days of age).

Contagious diseases must be taken into account when litters are mixed. Thus, when an early diarrheal disease appeared in Room B, it slowed the growth rate of the Mixed piglets at the beginning of the growth period. Pigs were not able to compensate for this after weaning, so overall ADG was lower in affected pigs. This effect was especially notable when piglets were mixed and diarrhea became more widespread: mixed piglets in an affected room had the lowest growth rate from birth to weaning.

In this study, the N:L ratio was affected by weaning but not by room or previous mixing. Other studies^{16,17} across a number of species have shown that stress conditions result in a redistribution of white blood cells, resulting in an increase in numbers of neutrophils (heterophils in poultry) and a decrease in numbers of lymphocytes, and thus a higher N:L ratio. Few studies have examined the effect of different housing treatments on the N:L ratio of piglets. Puppe et al,¹⁶ studying the effect of postweaning housing treatments (familiar or unfamiliar environment) and social conditions (pigs from the same litter or a different litter housed together), observed that the N:L ratio increased from 0.6 to 1.2 the day after weaning. There was a greater increase in pigs subjected to both an

unfamiliar environment and different social conditions, although the effect of the environment was greater than that of the social conditions. In any case, the increase in N:L ratio was transient, declining by the fourth day after weaning.

No differences were observed in mixing or room effects on the N:L ratio, despite the occurrence of diarrhea in Room B. Arriba et al¹⁸ reported a lymphocyte proliferative response against porcine epidemic diarrhea virus strain-CV777, and Jonasson et al¹⁹ showed that neutrophils and lymphocytes increased in pigs with swine dysentery, even during the recovery period. No published studies were identified in which the N:L ratio was either measured in pigs with diarrhea or related to *E. coli* diarrhea.

There was no effect of mixing on APP, but significant differences appeared between rooms. We found a higher concentration of APPs 2 days after weaning in Room B, where immunological stimulation had occurred because of *E. coli* infection, suggesting that affected animals had not yet completely recovered. In addition, diarrhea had the same effect on Mixed and Control pigs, but growth performance of affected Mixed pigs was more severely affected. It is possible that they were more immunologically challenged and were unable to mount an adaptive stress response to mixing. There was no effect of mixing on APP concentration in pigs in Room A. No references are available for comparison with our study regarding the effect of mixing piglets on APP concentration. However, it is established^{20,21} that APP concentrations increase as a result of inflammation caused by tissue damage or infection, and Jacobson et al²² reported that SAA and Hp both increased in pigs suffering from other digestive illnesses such as swine dysentery. A significant increase in APP has also been reported when cytokine production is stimulated orally by lipopolysaccharides.²³

Regarding the pattern of behavior, the pigs' predominant activity was resting (75.5% of the time), followed by eating (13.6%); walking, scratching, and rooting (5.4%); inactivity (3.1%); drinking (1.3%); excreting (0.6%); and fighting (0.5%). We observed that after weaning, pigs from Room B, where the outbreak of diarrhea had occurred, spent more time inactive, in agreement with their higher mean APP concentration 2 days post weaning, suggesting that recovery from diarrhea remained incomplete at this time.

Mixed pigs had more active behavior immediately after both mixing and weaning, spending more time alert, nosing, rooting, and fighting than the Controls. In agreement with these results, Parratt et al¹⁴ reported that when piglets were mixed 5 days prior to weaning, there was significantly more fighting in the preweaning period. However, these authors found that immediately post weaning, there was less fighting among mixed pigs than controls, although this difference disappeared over time. Also, Weary et al^{5,24} reported that pigs fought less at weaning when they had been mixed previously. Kanaan et al¹⁵ mixed piglets on day 13 after birth and analyzed their behavior on day 16 using three tests: social challenge, isolation, and backtest (which determines the coping behavior of a piglet in a standardized stress situation). Responses for isolation and backtest did not differ, but there was a longer latency to the first aggressive behavior, and shorter duration and lower frequency of aggressive interactions. Ear injuries were more abundant in mixed litters on day 15, but this difference disappeared by day 18. Thus, when piglets were socialized before weaning, there was less fighting behavior post weaning, suggesting that it was easier for these pigs to cope with the stress of weaning.

Implications

- Under the conditions of this study, mixing litters of piglets with *E coli* diarrhea may have a detrimental effect on growth rate.
- Pigs with colibacillosis may have higher serum concentrations of APPs, which are physiological stress markers.
- Piglets mixed with another litter prior to weaning spend less time fighting after weaning than do controls, which may help them cope with the stress of weaning.

Acknowledgements

The Department of Agricultural Science and Technology gratefully acknowledges the assistance of Juan Carlos Conesa Legaz in allowing this study to take place at his commercial swine production facility.

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