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Citrullinemia is a suitable biomarker for post weaning performance in piglets under intensive farming

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OBJECTIVE
To describe citrullinemia profiles during the weaning transition and correlate citrulline production with stress and growth in a commercial pig farm.

ANIMALS
240 healthy piglets of homogenous weight, weaned from second and third parity sows, were selected at weaning and subjected to the farm's routine management practices in May to July 2020 and May to July 2021.

PROCEDURES
Piglets were weighed at weaning, then 15 and 49 days later in order to calculate daily weight gain during the first 15 and 49 days after weaning. Blood samples were collected from each piglet to determine citrulline and cortisol profiles during the early postweaning period.

RESULTS
Citrullinemia decreased dramatically during the first week postweaning and then increased progressively to reach preweaning values by 15 days postweaning. Citrulline production during the first 2 weeks postweaning was negatively correlated with cortisol production (r: −0.2949) and positively correlated with mean daily weight gain during the first 15 (ρ: 0.5450) and 49 (ρ: 0.6603) days postweaning.

CLINICAL RELEVANCE
Citrullinemia profile of piglets during the early postweaning period showed a temporal negative impact of stress (assessed by plasmatic cortisol levels) on intestinal enterocytes' mass and function, which resulted in a lower average daily weight gain. We demonstrated that a single biomarker, plasmatic citrulline, is useful to describe intestinal metabolism during the early postweaning period and that the greater the citrulline production during the first days after weaning, the higher the weight gain during the entire postweaning period.

Weaning stress syndrome, associated with early weaning practices (3 to 4 weeks of age), is a common feature in intensive pig production systems. The sudden change from liquid to solid diet, a new environment, and the adaptation to a different social group inevitably induce stress, which negatively impacts gastrointestinal metabolism. This situation often leads to intestinal morphological and functional changes that impair nutrient absorption[1] and promote biochemical alterations in the gut-brain axis, resulting in abnormal behavior, depression, low voluntary feed intake or anorexia, and ultimately compromised performance.[2–4] Stress triggers the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the subsequent secretion of corticotrophin-releasing factor (CRF). Glucocorticoids, catecholamines, and CRF exert a direct action on the intestine, causing inflammation, decrease of immune system response, and reduction of functional intestinal mass. Cortisol is the

main glucocorticoid in pigs, and it has been shown to increase during the weaning transition as a consequence of stress.[5,6]

Structural and functional biomarkers can be used to study intestinal and systemic changes in response to weaning, as follows: enzymatic activity (intestinal epithelial disaccharidases, trypsin, plasmatic cholecystokinin, etc), sugars absorption, villi length, crypts depth, colonic and caecal bacteria, pH of stomach and intestinal contents, weight of the intestine and pancreas, genes transcription and regulation, production of acute phase proteins, and fecal calprotectin, among others.[7–12] In most cases, animals must be slaughtered at different time points and several parameters must be analyzed together to explain a physiologic situation. A single variable obtained by a minimally invasive intervention would be valuable to describe intestinal changes after weaning and predict growth performance by the end of the postweaning period.

Citrulline is a nonprotein amino acid entirely synthetized by the enterocytes in proximal segments of the small intestine.[13,14] Citrullinemia has been used in humans as a biomarker of intestinal metabolic activity and enterocytes' mass in various enteropathies like Crohn disease, villi atrophy, tropical enteropathy, mucositis, and acute rejection in intestinal transplantation.[15–17] In pigs, the precursors of citrulline are the most abundant dietary amino acids, L-glutamine and L-glutamate.[18,19] Upon its synthesis, citrulline is released through the basolateral membrane into the portal circulation[18] and bypasses the liver (as it is poorly incorporated into the hepatocytes) to enter systemic circulation.[20] In this way, citrullinemia reveals as a proper biomarker for monitoring intestinal function of piglets during the weaning transition. Berkeveld et al[8] determined that citrullinemia can be used as a longitudinal marker of intestinal function during the early postweaning period, but that study was performed under experimental farm conditions where the animals were not exposed to typical commercial farms' stressors such as high stocking densities (and large numbers of animals per pen rendering higher possibilities of fights to establish social rank), routine regrouping of animals, and exposure to different farm workers. Therefore, the purpose of the present work was to describe citrullinemia profiles during the weaning transition and correlate citrulline production with stress and growth in a commercial pig production farm.

Materials and Methods

This study was performed in a commercial pig production farm in Buenos Aires Province, Argentina. All animals were subjected to the farm's routine management practices in a farrow-to-finish production system. The farm possessed a high health status and no history of chronic diseases. Prophylactic antimicrobials were not used. The sows routinely received a combined erysipelas-parvovirus-leptospirosis vaccine 10 days after farrowing, and antiparasitic drugs (for internal and external parasites) were used before entering the farrowing room. The piglets received a cirvovirus and mycoplasma single-dose vaccine at 28 days of age. Porcine reproductive and respiratory syndrome, African swine fever, classical swine fever, and porcine epidemic diarrhea are not present in Argentina.[21,22]

Laboratory analyses were carried out at the Toxicology Laboratory of the Faculty of Veterinary Sciences, National University of the Center of the Buenos Aires Province-Veterinary Research Center of Tandil, Buenos Aires, Argentina. All procedures for animal handling were performed according to the Animal Welfare Committee of the National University of the Center of the Buenos Aires Province (Academic Council Resolution 087/02; internal protocol 16/2020) in compliance with EU Directive 2010/63/EU.

Animals and sampling

Two hundred and forty piglets (50% [120/240] castrated males and 50% [120/240] females) of homogenous weight (6.03 ± 0.97 kg), weaned from second or third parity sows of the same commercial genetic line (Swine Genetic Branch; Choice Genetics Co), were selected at weaning (20 ± 1 days). The trial was carried out in 12 replicates of 20 piglets each (6 replicates through mid-May to early July 2020 and 6 replicates through the same period in 2021). The selected animals were marked with a crayon as eligible. Seven eligible animals from each replicate were randomly designated for further sampling, tagged, and grouped with age-matched mates at 0.35 m^2/piglet in 25-m^2 rooms inside the same barn with full slatted floors. Environmental conditions were controlled by a computerized automatic ventilation system using air extractors, evaporative cooling pads, and heating lamps to maintain room temperature (29 °C for the first 7 days and lowered by 1 °C each week thereafter). Stainless steel wet-dry feeders and stainless steel nipple drinkers (1 nipple/10 piglets) allowed free access to feed and water. High digestibility protein decreased from 22% to 19% and metabolizable energy from 3,400 to 3,350 kcal/kg from the beginning to the end of the weaning stage (20 to 70 days of age). Lysine contents and all other nutrients were supplied in compliance with the National Research Council.[23] Each piglet was weighed at weaning and 15 and 49 days later to calculate mean daily weight gain during the first 15 (ADG_{15}) and 49 days (ADG_{49}) after weaning.

For blood sampling, the animals were restrained manually in supine position, supported in the handler's arms, carefully exposing the neck for venipuncture. Only 2 skilled veterinarians were authorized to carry out this procedure, which took 15 seconds at most. Serial samples were collected from tagged piglets to determine citrulline and cortisol profiles during the first 15 and 12 days postweaning, respectively. Blood samples were

collected from the external jugular vein at weaning (day 0) and 4, 8, 12, and 15 days later using a vacuum blood collection tube. Sampling began at 8:00 AM and took 30 minutes at most.

Plasma was obtained by centrifugation at 11,200 *g* for 10 minutes and stored at –20 °C until analyzed. Plasmatic cortisol concentrations were measured using a radioimmunoassay kit (IM 1841; Beckman Coulter Co) previously used with pig plasma.[24] The sensitivity of the assay was 5 nmol/L and the intra-assay coefficient of variation was 6.4% between 20 and 2,000 nmol/L. All samples were measured in duplicates.

Plasmatic citrulline concentrations were analyzed by high performance liquid chromatography with fluorescence detection according to the method described by Wu and Meininger.[25] Briefly, 100 µL plasma was taken into 2.0 mL centrifuge tubes and 200 µL of methanol was added for protein precipitation. After vortex mixing for 2 minutes, samples were centrifuged at 22,000 *g* for 10 minutes. The clear supernatant was filtered through 0.22 µm nylon membranes. For derivatization, 50 µL o-phthalaldehyde reagent was added to 50 µL of the supernatant and vortex mixed. The derivatization step took place 5 minutes before injection into the high performance liquid chromatography with fluorescence detection system. Separation was achieved on a C18 stationary phase column of 5 µm, 250 X 460 mm maintained at 30 °C. The mobile phase consisted of (A) sodium acetate buffer 50 mmol/L (pH 6.8) and (B) methanol:acetonitrile (2:1). A gradient elution method was used. Fluorescence detector was set at 338 and 425 nm for excitation and emission, respectively. The detector's response was linear over the range of 0.5 to 20 µmol/L, being r^2 coefficient > 0.999. The lower limit of quantification was 0.5 µmol/L. Accuracy (expressed as relative error) was 2.09%. Repeatability and intermediate precision were < 10% for all concentrations analyzed.

Statistical analysis

Citrulline and cortisol area under the concentration versus time curves (AUCcit [measured as µmol X day/L] and AUCcor [measured as nmol X day/L], respectively) were calculated with software for pharmacokinetics data noncompartmental analysis (PK Solutions version 2.0; Summit Research Services Co).[26] AUC is used in endocrinology to estimate hormonal changes and assess overall production over a period of time.[27]

Statistical analyses were performed with standard software (RStudio version 4.2.2; RStudio Inc).[28] Normality and homoscedasticity were assessed by Shapiro-Wilk and Barlett tests, respectively. The response variables plasmatic cortisol and citrulline concentrations were analyzed by repeated-measures ANOVA to evaluate sampling day effects. When ANOVA resulted in statistically significant effects, the Tukey test was used to evaluate differences between sampling days. Results from 1 pig that died before the end of the trial were not included in the analysis.

Pearson rank-order correlation (r) was used to evaluate normally distributed data, and Spearman rank-order correlation (ρ) was used to evaluate data that were not normally distributed to determine associations between the following variables: AUCcit and plasmatic citrulline concentration at each sampling day, AUCcit-AUCcor, AUCcit-ADG_{15}, AUCcit-ADG_{49}, plasmatic citrulline concentration 4 days postweaning (CIT4)-ADG_{15}, CIT4-ADG_{49}, plasmatic citrulline concentration 8 days postweaning (CIT8)-ADG_{15}, and CIT8-ADG_{49}. The level of statistical significance was expressed as $P < .05$.

Results

Results for plasmatic citrulline concentration on day 4 were as follows: AUCcit and AUCcor were normally distributed, and the remaining variables were not normally distributed. Effects of sampling day were observed for plasmatic citrulline ($P < .001$) and cortisol ($P < .001$) concentrations **(Table 1)**.

Table 1—Mean ± SD plasmatic citrulline and cortisol concentrations on postweaning days 0, 4, 8, 12, and 15 for 83 healthy piglets of homogenous weight, weaned from second and third parity sows of the same genetic line between May and July 2020 or May and July 2021 in an intensive pig production farm in Argentina.

Days postweaning	Plasmatic citrulline (µmol/L)	Plasmatic cortisol (nmol/L)
0	74.47 ± 15.42[a]	145.60 ± 40.48[a]
4	41.29 ± 14.73[b]	180.37 ± 55.47[b]
8	43.53 ± 19.56[b]	148.40 ± 60.42[a]
12	56.12 ± 23.05[c]	137.44 ± 45.83[a]
15	69.50 ± 20.92[a]	NA

NA = Not assessed.
[a–c]For each column, results with different letters differed significantly ($P < .05$).

Mean plasmatic citrulline concentrations decreased rapidly and dramatically during the early weaning period (up to 8 days postweaning) but raised again toward 12 days postweaning to reach preweaning values by 15 days after weaning. Mean ± SD AUCcit was 750 ± 180. AUCcit was positively correlated with CIT4 (r: 0.6941; $P < .001$) and CIT8 (ρ: 0.7096; $P < .001$; **Figure 1**). No correlation was obtained between AUCcit and citrulline concentration on day 0, 12, or 15 postweaning.

On the contrary, plasmatic cortisol levels increased significantly 4 days after weaning and fell to similar weaning values 8 and 12 days later. The mean ± SD value obtained for AUCcor was 1975 ± 585. A negative correlation was found between AUCcit and AUCcor (r: –0.2949; $P = .0210$; **Figure 2**).

The piglets weighed 11.41 ± 2.13 kg and 27.20 ± 4.57 kg 15 and 49 days postweaning, respectively. Both ADG_{15} and ADG_{49} were as typically expected in the present production system, with mean values of 0.38 ± 0.12 and 0.44 ± 0.08, respectively.

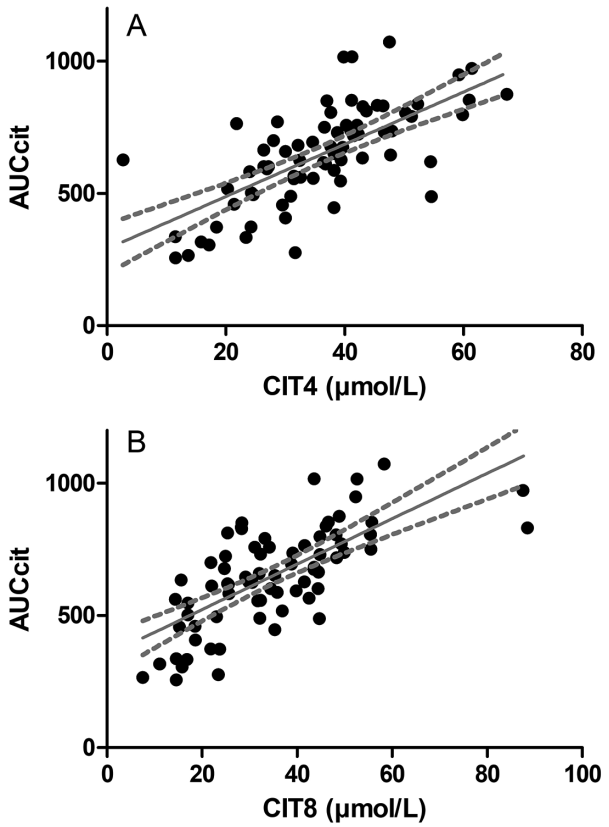

Figure 1—Correlations between area under plasmatic citrulline concentration-versus-time curve (AUCcit, measured as µmol X day/L) and plasmatic citrulline concentration on day 4 postweaning (CIT4; r = 0.6941; *P* < .001; A) and on day 8 postweaning (CIT8; ρ = 0.7096; *P* < .001; B) for 83 healthy piglets of homogenous weight, weaned from second or third parity sows of the same genetic line between May and July 2020 or May and July 2021 in an intensive production farm in Argentina. The solid line represents the correlation line, the dotted lines represent the 95% CI of the correlation line, and each circle represents the results for 1 animal.

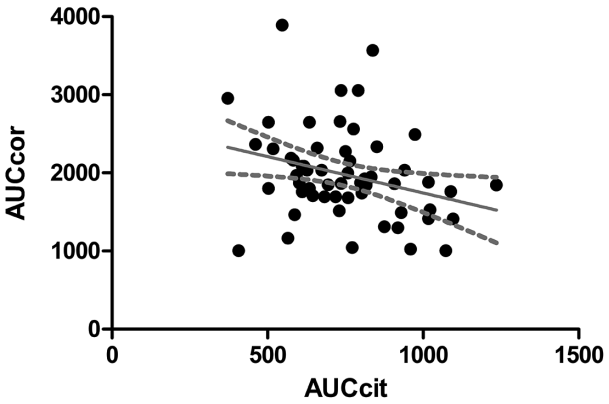

Figure 2—Correlation between plasmatic cortisol area under concentration-versus-time curve (AUCcor, measured as nmol X day/L) and AUCcit during postweaning days 0 to 15 (r = –0.2949; *P* = .0210) for the piglets described in Figure 1.

Regarding correlations between citrullinemia and performance parameters, we observed that ADG_{15} was positively correlated with AUCcit (ρ: 0.5450; *P* < .001), CIT4 (ρ: 0.2716; *P* = .0262) and CIT8 (ρ: 0.4112; *P* < .001; **Figure 3**) and that ADG_{49} was positively correlated with AUCcit (ρ: 0.6603; *P* < .001), CIT4 (ρ: 0.5276; *P* < .001) and CIT8 (ρ: 0.4956; *P* < .001; **Figure 4**).

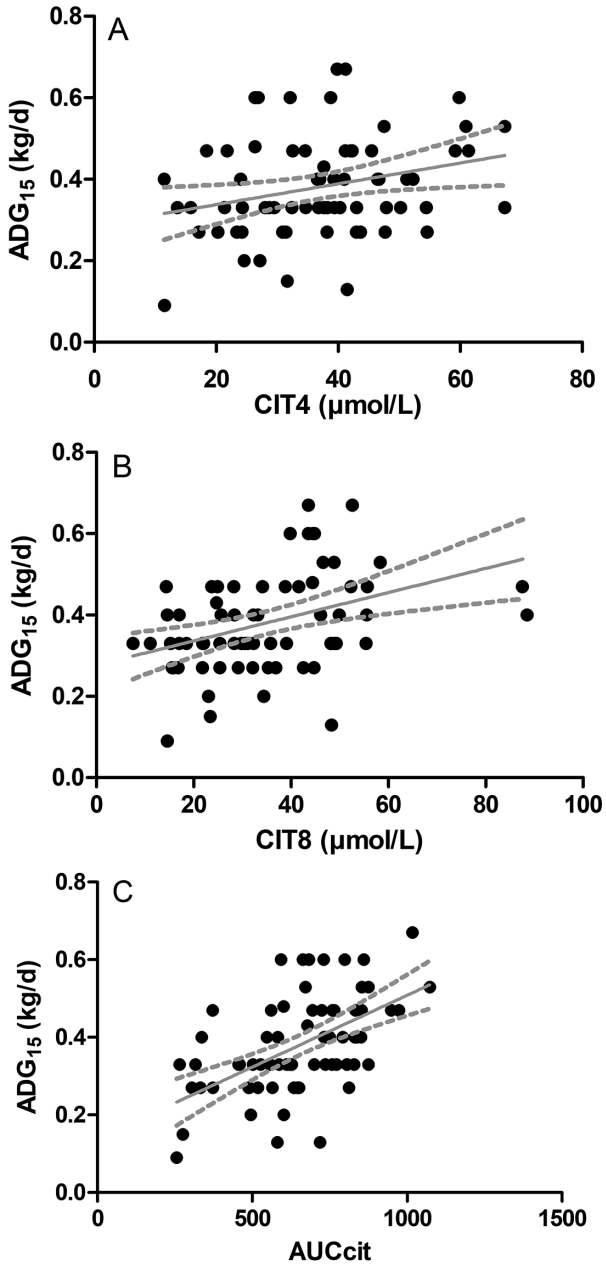

Figure 3—Correlations between average daily weight gain 15 days postweaning (ADG_{15}) and CIT4 (ρ: 0.2716; *P* = .0262; A), CIT8 (ρ: 0.4112; *P* < .001; B), and AUCcit (ρ: 0.5450; *P* < .001; C) for the piglets described in Figure 1.

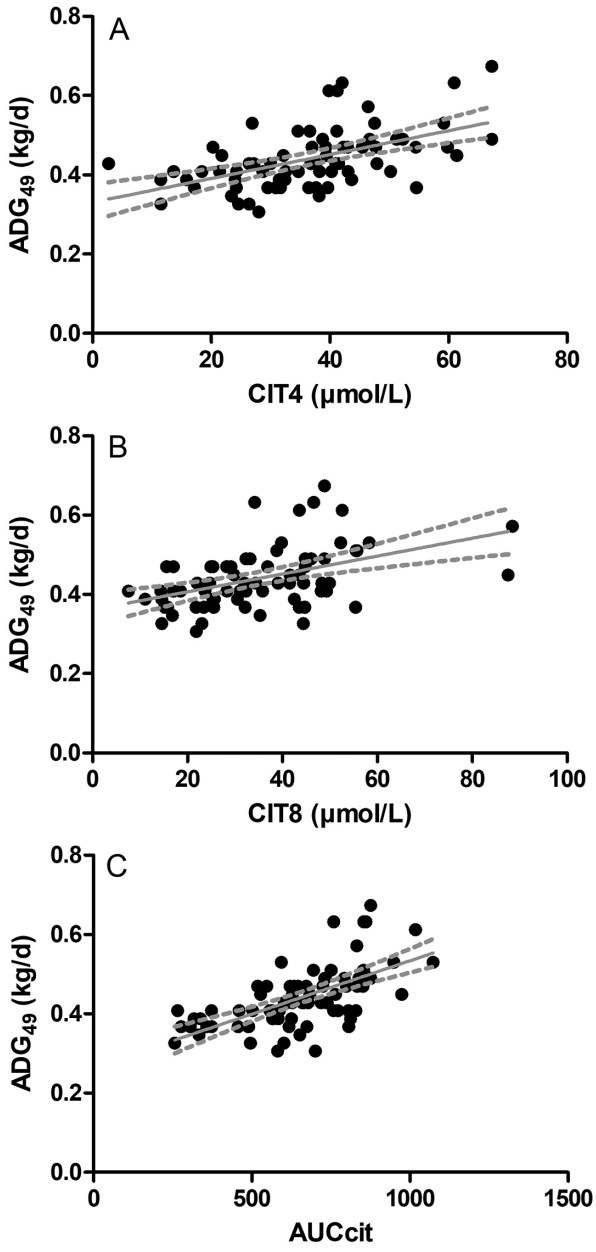

Figure 4—Correlations between average daily weight gain 49 days postweaning (ADG_{49}) and CIT4 (ρ: 0.5276; $P < .001$; A), CIT8 (ρ: 0.4956; $P < .001$; B), and AUCcit (ρ: 0.6603; $P < .001$; C) for the piglets described in Figure 1.

Discussion

In the present work, we studied the temporal changes of plasmatic citrulline concentrations associated with early weaning in an intensive pig production system. Our results showed an abrupt decrease in citrullinemia during the first 4 to 8 days postweaning, followed by a gradual increase to reach preweaning values by 15 days postweaning. In previous studies, we obtained similar citrullinemia profiles in weaned piglets that received different diets.[29] These events are consistent with Montagne et al,[10] who described detrimental changes in the gut architecture and function associated to weaning during an acute and short time period (up to 5 days postweaning) followed by a gradual adaptative and maturational phase. Also, in agreement with our findings, Berkeveld et al[8] showed that weaned piglets had lower plasmatic citrulline concentrations than their unweaned littermates during the first week postweaning with the nadir 4 days postweaning. This effect can be explained by the impact of weaning stress on intestinal metabolism and functional enterocytes' mass. The activation of the HPA axis toward stress responses produces negative intestinal changes associated with an increase of plasmatic CRF; production of cortisol, adrenalin, and noradrenaline; oxidative stress; intestinal mast cell degranulation and release of inflammatory mediators; detrimental effects on the immune system; and dysbiosis.[3,6,30–33] At the same time, the piglets experience a dramatic reduction in nutritional intake[34] and stress-induced anorexia develops in most of newly weaned piglets. According to Brooks et al,[35] while 50% of the piglets take their first feed 24 hours after weaning, approximately 10% do not eat up to more than 48 hours later. Untoward results of these processes are reduced villi height, increased crypts depth, reduced enterocytes mass, and decrease of nutrient availability (including the amino acids glutamine, glutamate, and proline, which are precursors of citrulline synthesis) as a consequence of anorexia.

The increase in plasmatic cortisol concentration, which is related to the HPA axis activation, has been widely used as a biomarker of stress in pigs. The cortisol surge that we observed during the initiation of the postweaning period in the present study is in agreement with other investigations.[29,36,37] Noteworthy, even though Wu et al[37] demonstrated that augmented cortisol levels after weaning enhance the transcription of pyrroline-5-carboxylate synthase mRNA and therefore the capacity of enterocytes for citrulline synthesis from glutamine, it is possible that the lack of substrates overrides the improved enzymatic activity. The upregulation of citrulline biosynthetic pathway by cortisol may be an adaptative response to the lack of nutrient absorption during stressful situations. The large variation of plasmatic citrulline and cortisol concentrations between individuals of the same group in the present study can be linked to the way each individual responds to weaning stress, expressing different modifications in the structure and functioning of the intestine.[38] In this regard, different authors demonstrated great variability in absorption and other pharmacokinetic parameters of oral antimicrobials rendering variable therapeutic effects.[39–43]

As we observed in the present study, overall citrulline production during early weaning period, represented by AUCcit, negatively correlated with overall plasmatic cortisol production, represented by AUCcor, providing further evidence of the detrimental impact of stress on intestinal metabolism. In line with these findings, higher citrullinemia during the

early postweaning period indicates a better intestinal function, which in practice reflects in higher productive performance, as demonstrated by the positive correlation between AUCcit and ADG from weaning to 15 and 49 days later. Moreover, we have shown that citrullinemia measured on a single day during the acute phase of postweaning stress (day 4 or 8 postweaning in our study) can be used as an indicator of growth performance along the entire postweaning period. This information would be useful to evaluate groups of problematic piglets (eg, presenting diarrhea or another stressful situation) with only 1 blood sample from a representative number of animals and, if necessary, arrange nutritional or management interventions to compensate for detrimental effects of gastrointestinal disorders after weaning.

Our results were limited to the described production system (complete cycle) and genetic line. Moreover, the work was performed in a medium-size commercial farm, with high sanitary standards and trained personnel that had been working in the farm as a team for at least 10 years, which created a rather ideal scenario to maintain good animal husbandry practices and minimum stress. The reality of other types of production systems (wean-to-finish), genetics, or management (like routine use of prophylactic antimicrobials or lack of creep feeding) may render different results. For example, we can speculate that in highly stressed animals (presenting aggressive behavior or high prevalence of infections) plasmatic citrulline would decrease after weaning and maintain low concentrations throughout the sampling period. In addition, for our study we selected litters from second or third parity sows, which are less prone to present farrowing difficulties, produce higher amounts of colostrum of better quality, and show better mothering ability than gilts or old animals.[44] In this way, the selected litters would have benefited from a higher colostrum intake providing higher concentrations of maternal antibodies and mitogenic peptides (that trigger the development of the gastrointestinal tract) than litters from gilts or old animals. At weaning, higher levels of maternal antibodies and a more developed gastrointestinal tract would cushion the impact of stress on the gastrointestinal tract.

In the present work we demonstrated that a single biomarker, plasmatic citrulline, is useful to describe intestinal metabolism and function during the early postweaning period and established the relationship between citrullinemia and productive performance in commercial pig farming. In this way, citrulline production appears as an appropriate biomarker to identify intestinal functional responses to changes in diet composition, incorporation of feed additives or therapeutic treatments.

Acknowledgments

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The authors declare that there were no conflicts of interest.

References

1. Lallès J-P, Boudry G, Favier C, et al. Gut function and dysfunction in young pigs: physiology. *Anim Res*. 2004;53(4):301–316. doi:10.1051/animres:2004018
2. Cryan JF, O'Riordan KJ, Cowan CSM, et al. The microbiota-gut-brain axis. *Physiol Rev*. 2019;99(4):1877–2013. doi:10.1152/physrev.00018.2018
3. Kobek-Kjeldager C, Schönherz AA, Canibe N, Pedersen LJ. Diet and microbiota-gut-brain axis in relation to tail biting in pigs: a review. *Appl Anim Behav Sci*. 2022;246:105514. doi:10.1016/j.applanim.2021.105514
4. Pollock J, Glendinning L, Smith LA, et al. Temporal and nutritional effects on the weaner pig ileal microbiota. *Anim Microbiome*. 2021;3(1):58. doi:10.1186/s42523-021-00119-y
5. Li LA, Yang JJ, Li Y, et al. Effect of weaning age on cortisol release in piglets. *Genet Mol Res*. 2016;15(2): doi:10.4238/gmr.15027693
6. Pluske JR, Miller DW, Sterndale SO, Turpin DL. Associations between gastrointestinal-tract function and the stress response after weaning in pigs. *Anim Prod Sci*. 2019;59(11):2015–2022. doi:10.1071/AN19279
7. Aardsma MP. *Alternative measures of physiological stress in nursery pigs and broiler chickens*. Doctoral dissertation. Purdue University Graduate School; 2019.
8. Berkeveld M, Langendijk P, Verheijden JHM, et al. Citrulline and intestinal fatty acid-binding protein: longitudinal markers of postweaning small intestinal function in pigs? *J Anim Sci*. 2008;86(12):3440–3449. doi:10.2527/jas.2008-1167
9. Bogere P, Choi YJ, Heo J. Optimization of fecal calprotectin assay for pig samples. *J Agric Life Sci*. 2019;53(1):93–104. doi:10.14397/jals.2019.53.1.93
10. Montagne L, Boudry G, Favier C, Le Huërou-Luron I, Lallès JP, Sève B. Main intestinal markers associated with the changes in gut architecture and function in piglets after weaning. *Br J Nutr*. 2007;97(1):45–57. doi:10.1017/S000711450720580X
11. Slinger KR, Stewart AH, Daniel ZCTR, et al. The association between faecal host DNA or faecal calprotectin and feed efficiency in pigs fed yeast-enriched protein concentrate. *Animal*. 2019;13(11):2483–2491. doi:10.1017/S1751731119000818
12. Bučková K, Muns R, Cerón J, Kyriazakis I. Consequences of timing of organic enrichment provision on pig performance, health and stress resilience after weaning and regrouping. *Animal*. 2022;16(10):100637. doi:10.1016/j.animal.2022.100637
13. Curis E, Nicolis I, Moinard C, et al. Almost all about citrulline in mammals. *Amino Acids*. 2005;29(3):177–205. doi:10.1007/s00726-005-0235-4
14. Windmueller HG, Spaeth AE. Source and fate of circulating citrulline. *Am J Physiol*. 1981;241(6):E473–E480. doi:10.1152/ajpendo.1981.241.6.E473
15. Crenn P, Messing B, Cynober L. Citrulline as a biomarker of intestinal failure due to enterocyte mass reduction. *Clin Nutr*. 2008;27(3):328–339. doi:10.1016/j.clnu.2008.02.005
16. Papadia C, Kelly P, Caini S, et al. Plasma citrulline as a quantitative biomarker of HIV-associated villous atrophy in a tropical enteropathy population. *Clin Nutr*. 2010;29(6):795–800. doi:10.1016/j.clnu.2010.04.008
17. van der Velden WJFM, Herbers AHE, Brüggemann RJM, Feuth T, Peter Donnelly J, Blijlevens NM. Citrulline and albumin as biomarkers for gastrointestinal mucositis in recipients of hematopoietic SCT. *Bone Marrow Transplant*. 2013;48(7):977–981. doi:10.1038/bmt.2012.278
18. Wu G. Synthesis of citrulline and arginine from proline in enterocytes of postnatal pigs. *Am J Physiol*. 1997;272(6 pt 1):G1382e90. doi:10.1152/ajpgi.1997.272.6.G1382

19. Wu G, Knabe DA, Flynn NE. Synthesis of citrulline from glutamine in pig enterocytes. *Biochem J*. 1994;299(pt 1):115e21. doi:10.1042/bj2990115
20. Maric S, Restin T, Muff JL, et al. Citrulline, biomarker of enterocyte functional mass and dietary supplement. Metabolism, transport, and current evidence for clinical use. *Nutrients*. 2021;13(8):2794. doi:10.3390/nu13082794
21. Vigilancia epidemiologica para peste porcina clasica (PPC) y sindrome respiratorio reproductivo porcino (PRRS) en cerdos domesticos 2021. Programa de Enfermedades de los Porcinos – DPYESA – DNSA – SENASA. Informe Técnico. Accessed February 23, 2023. https://www.argentina.gob.ar/sites/default/files/informe_muestreo_porcino_2021.pdf
22. Diarrea epidémica porcina (DEPv). Ministerio de Economía, Agricultura Ganadería y Pesca-SENASA. Accessed February 23, 2023. https://www.argentina.gob.ar/senasa/programas-sanitarios/cadenaanimal/porcinos/porcinos-produccion-primaria/diarrea-epidemica-porcina-depv
23. National Research Council; Division on Earth and Life Studies; Board on Agriculture and Natural Resources; Committee on Nutrient Requirements of Swine. *Nutrient Requirements of Swine*. 11th ed. The National Academies Press; 2012.
24. Pluschke AM, Williams BA, Zhang D, Anderson ST, Roura E, Gidley MJ. Male grower pigs fed cereal soluble dietary fibres display biphasic glucose response and delayed glycaemic response after an oral glucose tolerance test. *PLoS One*. 2018;13(3):e0193137. doi:10.1371/journal.pone.0193137
25. Wu G, Meininger CJ. Analysis of citrulline, arginine, and methylarginines using high-performance liquid chromatography. *Methods Enzymol*. 2008;440:177–189. doi:10.1016/S0076-6879(07)00810-5
26. Farrier DS. *PK solutions: a noncompartmental pharmacokinetic data analysis program*. Version 2.0.2. Summit Research Services; 1997.
27. Golden SH, Sánchez BN, Wu M, et al. Relationship between the cortisol awakening response and other features of the diurnal cortisol rhythm: the Multi-Ethnic Study of Atherosclerosis. *Psychoneuroendocrinology*. 2013;38(11):2720–2728. doi:10.1016/j.psyneuen.2013.06.032
28. *Integrated development for R*. RStudio Inc; 2015. Accessed April 20, 2020. https://www.Rstudio.com
29. Dieguez SN, Decundo JM, Martínez G, et al. Effect of dietary oregano (*Lippia origanoides*) and clover (*Eugenia caryophillata*) essential oils' formulations on intestinal health and performance of pigs. *Planta Med*. 2022;88(03/04):324–335.
30. Boudry G, Péron V, Le Huërou-Luron I, Lallès JP, Sève B. Weaning induces both transient and long-lasting modifications of absorptive, secretory, and barrier properties of piglet intestine. *J Nutr*. 2004;134(9):2256–2262. doi:10.1093/jn/134.9.2256
31. Campbell JM, Crenshaw JD, Polo J. The biological stress of early weaned piglets. *J Anim Sci Biotechnol*. 2013;4(1):19. doi:10.1186/2049-1891-4-19
32. Petrosus E, Silva EB, Lay D Jr, Eicher SD. Effects of orally administered cortisol and norepinephrine on weanling piglet gut microbial populations and *Salmonella* passage. *J Anim Sci*. 2018;96(11):4543–4551.
33. Smith F, Clark JE, Overman BL, et al. Early weaning stress impairs development of mucosal barrier function in the porcine intestine. *Am J Physiol Gastrointest Liver Physiol*. 2010;298(3):G352–G363. doi:10.1152/ajpgi.00081.2009
34. Weng RC. Dietary fat preference and effects on performance of piglets at weaning. *Asian-Australas J Anim Sci*. 2017;30(6):834–842. doi:10.5713/ajas.16.0499
35. Brooks PH, Moran CA, Beal JD, Demeckova V, Campbell A. Liquid feeding for the young piglet. In: Varley MA, Wiseman J, eds. *The Weaner Pig: Nutrition and Management*. CAB International; 2001:153–178.
36. Moeser AJ, Klok CV, Ryan KA, et al. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. *Am J Physiol Gastrointest Liver Physiol*. 2007;292(1):G173–G181. doi:10.1152/ajpgi.00197.2006
37. Wu G, Meininger CJ, Kelly K, Watford M, Morris SM Jr. A cortisol surge mediates the enhanced expression of pig intestinal pyrroline-5-carboxylate synthase during weaning. *J Nutr*. 2000;130(8):1914–1919. doi:10.1093/jn/130.8.1914
38. Xun W, Shi L, Zhou H, Hou G, Cao T. Effect of weaning age on intestinal mucosal morphology, permeability, gene expression of tight junction proteins, cytokines and secretory IgA in Wuzhishan mini piglets. *Ital J Anim Sci*. 2018;17(4):976–983. doi:10.1080/1828051X.2018.1426397
39. Ferraris RP, Carey HV. Intestinal transport during fasting and malnutrition. *Annu Rev Nutr*. 2000;20(1):195–219. doi:10.1146/annurev.nutr.20.1.195
40. Soler C, Goossens T, Bermejo A, et al. Digestive microbiota is different in pigs receiving antimicrobials or a feed additive during the nursery period. *PLoS One*. 2018;13(5):e0197353. doi:10.1371/journal.pone.0197353
41. Soraci AL, Amanto F, Tapia MO, de la Torre E, Toutain PL. Exposure variability of fosfomycin administered to pigs in food or water: impact of social rank. *Res Vet Sci*. 2014;96(1):153–159. doi:10.1016/j.rvsc.2013.12.003
42. Vinarov Z, Abdallah M, Agundez JAG, et al. Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: an UNGAP review. *Eur J Pharm Sci*. 2021;162:105812. doi:10.1016/j.ejps.2021.105812
43. Wijtten PJ, van der Meulen J, Verstegen MW. Intestinal barrier function and absorption in pigs after weaning: a review. *Br J Nutr*. 2011;105(7):967–981. doi:10.1017/S0007114510005660
44. Devillers N, Farmer C, Le Dividich J, Prunier A. Variability of colostrum yield and colostrum intake in pigs. *Animal*. 2007;1(7):1033–1041. doi:10.1017/S175173110700016X