

# Supplementation with coffee husk enhances lactational performance of piglets and IgG concentration in sow colostrum: A prepartum intervention

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## ABSTRACT

**Objective:** This study aimed to evaluate the effects of prepartum supplementation with coffee by-products specifically coffee husks on piglet performance, with a focus on weight gain and immunoglobulin G (IgG) concentrations in colostrum and milk.

**Design/methodology:** Coffee by-products were collected, dried, ground, and sieved, and caffeine content was quantified using high-performance liquid chromatography (HPLC). Between gestation days 110 and 112, 50 g of coffee husks mixed with feed were administered to the sows, delivering a total caffeine dose of 750 mg. Farrowing was induced on day 113 using cloprostenol. Piglets were weighed at birth and at weaning, and pre-weaning mortality was recorded. Additionally, colostrum and milk samples were analyzed for IgG concentrations using a Brix refractometer.

**Results:** While no significant differences were observed in birth weight, piglets from caffeine-supplemented sows exhibited significantly higher weaning weights and increased IgG concentrations in both colostrum and milk, indicating improved lactogenesis. Pre-weaning mortality was also markedly lower in the supplemented group (0.97%) compared to the control group (10.83%), suggesting enhanced neonatal vitality and reduced mortality risk. The incorporation of coffee by-products into animal diets offers potential sustainability and health benefits; however, possible anti-nutritional effects and regulatory challenges must be addressed for widespread application.

**Conclusions:** Prepartum supplementation of sows with coffee by-products enhances IgG levels in colostrum and milk, supporting the development of heavier piglets with reduced mortality. These outcomes contribute to improved productivity and profitability in small-scale swine production systems.

**Keywords:** Coffee husk, neonatal mortality, weaned piglets, caffeine.



## INTRODUCTION

Coffee is one of the most widely consumed commodities worldwide and ranks second only to petroleum in terms of raw material trade. Despite its global economic importance, coffee production generates over 23 million tons of by-products annually, primarily from the processing of coffee cherries into green coffee beans (Iriundo-DeHond *et al.*, 2020; Durán-Aranguren *et al.*, 2021; Lee *et al.*, 2023). These by-products including husks, pulp, parchment, mucilage, and silver skin are often discarded, contributing significantly to environmental waste. As approximately 90% of the coffee cherry becomes waste, this presents a critical environmental challenge and highlights the need for sustainable solutions, such as the valorization of these by-products through biotechnological applications (Iriundo-DeHond *et al.*, 2020; Klingel *et al.*, 2020; Bobková *et al.*, 2022). Coffee by-products are rich in bioactive compounds, including polyphenols, alkaloids, chlorogenic acid, antioxidants, carbohydrates, proteins, and dietary fiber. However, despite their high nutritional value, these compounds are often underutilized, exacerbating their environmental impact (Esquivel and Jiménez, 2012; Heeger *et al.*, 2016). In some cases, coffee by-products are repurposed as fertilizers or additives in animal feed. Nevertheless, their use as exclusive feed ingredients is limited due to the presence of anti-nutritional factors such as caffeine, tannins, and polyphenols which can reduce feed intake, impair nutrient absorption, and negatively affect weight gain in livestock species including tilapia, ruminants, and poultry (Durán-Aranguren *et al.*, 2021; Eckhardt *et al.*, 2022; Lee *et al.*, 2023). While the use of coffee by-products in monogastric animals, such as pigs, remains underexplored, existing research suggests caffeine may offer benefits in these species. Studies involving oral and injectable pharmaceutical-grade caffeine have shown its prophylactic and therapeutic potential in sows and their offspring. Doses ranging from 250 mg/sow/day to 6.4 g/sow have been administered without inducing nervous or behavioral disturbances, yielding improvements in thermoregulation, neonatal survival, lactation weight gain, gas exchange at birth, and overall weight gain during lactation especially in low-birth-weight piglets when combined with glucose (Superchi *et al.*, 2013; Dearlove *et al.*, 2018; Sanchez-Salcedo *et al.*, 2019; Jarrat *et al.*, 2023). Importantly, no adverse effects on maternal behavior were reported (Superchi *et al.*, 2016). The processing of 1 kg of coffee cherries produces approximately 430 g of by-products, which can contain between 3.4 and 18 mg of caffeine per gram. It is estimated that nearly 40% of the total caffeine is removed during processing and ends up in these by-products (Heeger *et al.*, 2016), making materials such as coffee husk a potentially sustainable source of caffeine.

Therefore, the aim of this study was to evaluate the effects of prepartum supplementation with coffee husk on piglet weight gain and immunoglobulin G (IgG) levels in colostrum and milk during parturition. We hypothesize that the caffeine present in coffee husk can enhance piglet health and performance, while also providing a sustainable strategy for repurposing waste from the coffee industry.

## MATERIALS AND METHODS

The experiment was conducted within a smallholder pig production system located in the central region of Veracruz, Mexico. A total of 223 piglets born to 20 multiparous sows

of hybrid Yorkshire-Landrace genetics (average weight:  $189.53 \pm 4.53$  kg; body condition score: 3; average previous litter size:  $13.5 \pm 1$  piglets) were included in the study. The sows were randomly assigned to two experimental groups: Control (n=11) and Coffee Husk (n=9). Group allocation was based on a prior statistical power analysis to ensure balanced baseline characteristics and sufficient sample sizes for detecting significant differences. All sows were multiparous (mean parity:  $2.66 \pm 0.12$ ). Individuals with compromised health or any condition likely to affect experimental outcomes were excluded. To minimize variability, 223 piglets were selected based on litter homogeneity within each group. Sows were housed individually in farrowing crates (1.5 m wide  $\times$  2.2 m long) beginning one week prior to the expected farrowing date. Environmental conditions were controlled, maintaining a temperature of  $27.8 \pm 0.46$  °C and relative humidity between 60-70%. Each farrowing crate was equipped with a piglet nest area containing an infrared heat lamp to ensure a postnatal temperature of 30 °C. All sows received a standard diet containing 13% crude protein, 3.2 Mcal ME/kg, and 18.6 g/day of lysine, with ad libitum access to water. Farrowing was induced in accordance with standard farm management practices, and all piglets were allowed immediate access to colostrum after birth. The experimental protocol was reviewed and approved by the Bioethics Committee of the Academic Program in Agricultural and Livestock Development at Universidad Veracruzana (approval code: MDA-BIO-002). All procedures were conducted in compliance with established national ethical guidelines.

### Sampling and analysis

**Coffee Husk Preparation:** The coffee husk used in this study was obtained via the wet processing method from a *Coffea robusta* plantation located in Cosautlán, Veracruz, Mexico (19° 20' 00" N, 96° 59' 00" W). After collection, the husk was sun-dried for three days and subsequently oven-dried at 40 °C until a constant weight was achieved. The dried material was ground using an electric mill (Hamilton Beach model CM08) and sieved through a 500- $\mu$ m mesh. The processed husk was then vacuum-sealed and stored in biosafety chambers to prevent contamination.

**Reagents:** Analytical-grade reagents used included acetonitrile (HPLC grade, 99.9%), formic acid (HPLC grade, 98-100%), methanol (HPLC grade, 99.8%), and ethanol (99.5%, Tedia Brand, Control Técnico y Representaciones, S.A. de C.V., Monterrey, Nuevo León, Mexico). HPLC-grade water (18 m $\Omega$ ) was produced using a Milli-Q50 purification system (Millipore Corp., Bedford, MA, USA). Authentic standards of alkaloids and phenolic compounds commonly found in coffee and its by-products were purchased from Sigma-Aldrich, with the following purities: caffeine (99%), trigonelline (97.5-102.5%), theobromine (99%), theophylline (99%), chlorogenic acid (95%), neochlorogenic acid (>98%), 3,5-dicaffeoylquinic acid (95%), 3,4-di-O-caffeoylquinic acid (90%), protocatechuic acid (97%), and caffeic acid (98%).

**Extraction Conditions:** To determine the chemical composition of the coffee husk and quantify caffeine content, Soxhlet extraction was performed. A 12.5 g sample of coffee husk was refluxed with 250 mL of ethanol (solid/liquid ratio: 1:20) at its boiling point (78 °C) for 7 hours. Ethanol was selected as the solvent based on its proven effectiveness in

caffeine extraction over methylene chloride (Román-Montalvo *et al.*, 2024). The extraction process was carried out in triplicate. Resulting extracts were evaporated to dryness and analyzed via HPLC.

**HPLC-DAD Analysis:** The dried ethanolic extract was analyzed using a Varian ProStar 320 HPLC system (Polaris Series) equipped with a diode array detector (DAD) and a C18 HYPERSIL ODS column (125×4.6 mm, 5 μm particle size), maintained at 30 °C. The mobile phase consisted of solvent A (0.1% aqueous formic acid) and solvent B (acetonitrile), both filtered through a 0.45 μm membrane and sonicated for 10 minutes. A gradient elution program was employed: 5% B (0-2 min), 7% B (2-4 min), 10% B (4-8 min), 12% B (8-12 min), 13% B (12-16 min), 14% B (16-20 min), 16% B (20-22 min), 18% B (22-24 min), 22% B (24-26 min), 26% B (26-28 min), 28% B (28-30 min), 30% B (30-32 min), and 100% B (32-44 min), at a flow rate of 1.0 mL/min.

The alkaloids and phenolic compounds detection were performed at 280 and 340 nm. Metabolite identification was based on retention time and spectral comparison with authentic standards. Caffeine quantification was conducted using an external calibration curve, with results expressed in mg of caffeine per kg of dried coffee husk. The linearity of the method was assessed using the  $R^2$  coefficient, calculated by analyzing the relationship between caffeine concentration (0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.10 mg/mL) and the corresponding peak area.

**Method Validation:** Linearity was assessed by plotting peak area against caffeine concentrations ranging from 0.01 to 0.10 mg/mL, and calculating the coefficient of determination ( $R^2$ ). Detection limit (DL) and quantification limit (QL) were established based on signal-to-noise ratios of 3 and 10, respectively, following Rada-Mendoza & Salazar S. (2011). Precision was determined by evaluating the repeatability of three area readings for a 0.05 mg/mL caffeine standard.

On the other hand, the accuracy was estimated in terms of the percentage of caffeine recovery (%R), similar to the method proposed by Tuesta-Hidalgo *et al.* (2024), but with some modifications. Briefly, 50 mg of caffeine standard (Caf) was added to 12.5 g of a coffee husk sample (fortified sample), which was then extracted using a Soxhlet, as described previously. The procedure was performed in duplicate. The result was calculated with the following equation:

$$\%R = \frac{\text{Quant. of Caf in the fortified sample} - \text{Natural Quant. of Caf}}{\text{Added amount of Caf}} \times 100$$

### Treatment administration

On day 110 of gestation (four days before the expected farrowing date on day 114), between 18:00 and 20:00 hours, 50 g of coffee husk mixed with the standard diet comprising broken yellow corn, sorghum, and commercial additives was offered to the sows for *ad libitum* consumption. This supplementation protocol was repeated on days 111 and 112, resulting in a total intake of 150 g of coffee husk and an estimated cumulative caffeine dose of 750 mg. On day 113, farrowing was induced at 9:00 a.m.

via intramuscular administration of 1 mL of cloprostenol (0.075 mg; InducelActive, Virbac, Mexico).

**Litter Weighing and Pre-Weaning Mortality:** All piglets were weighed immediately after birth and then returned to their respective pens. A second weighing was conducted at weaning, 21 days postpartum, using a mechanical floor scale. Pre-weaning mortality was calculated as the number of piglets that died before weaning divided by the number of live-born piglets, multiplied by 100 (Schodl *et al.*, 2019). After this, piglets were weaned, ear-tagged, and transferred to the weaning area, where they were grouped by weight following standard farm procedures.

**Colostrum and Milk Density Analysis:** Colostrum samples were collected manually approximately six hours after parturition from all teats in both the upper and lower rows of each sow. Samples were analyzed immediately using a Brix refractometer (Atago 3810 Pal-1, China), calibrated with distilled water before each set of measurements. A drop of colostrum was placed on the prism, and the Brix percentage was recorded to estimate IgG concentration, as described by Balzani *et al.* (2016). The same procedure was repeated for milk samples collected 24 hours postpartum.

### Statistical analysis

The analysis began with the sorting and classification of data obtained from the experimental procedures. Descriptive statistics and independent-sample t-tests were used to evaluate differences between groups, with birth weight, weaning weight, Brix percentage of colostrum and milk, and pre-weaning mortality serving as the dependent variables. A significance level of  $P \leq 0.05$  was established, and results were expressed as mean  $\pm$  standard error. Statistical analyses were conducted using the SigmaPlot 12 software package.

## RESULTS AND DISCUSSION

The use of coffee by-products as a food supplement for both human and animal consumption is not a novel concept. Initiatives in Europe and the Americas have been proposed to address the substantial volume of waste produced by the global coffee industry, which generates more than 23 million tons annually and is considered highly polluting (Eckhardt *et al.*, 2022). In this context and given the apparent lack of interest from major transnational corporations in effectively utilizing waste from coffee production systems (Rivera-Rojo, 2022) small-scale production systems present a viable alternative. To enhance their productivity and profitability, these systems must leverage accessible resources through a systems-based approach. Integrating surrounding agricultural activities can provide additional inputs or innovative methods to support farm operations (Ángel-Hernández *et al.*, 2021).

### Chemical coffee husk characterization

Six phenolic compounds and four alkaloids were identified in the chromatogram of the ethanolic extract of coffee husk: chlorogenic acid, neochlorogenic acid, 3,5-dicaffeoylquinic acid, 3,4-di-O-caffeoylquinic acid, caffeic acid, protocatechuic acid, theobromine, theophylline, trigonelline and caffeine. The calibration curve equation to

quantify caffeine was  $y=819052X-3778.4$  ( $R^2=0.9969$ ). DL and QL of the method were 0.011 and 0.038 mg/mL, respectively, whereas %R was  $91.18 \pm 3.37$  and precision was 2.08 %. The amount of caffeine found in the sample was  $4,962.67 \pm 816.52$  (mg of caffeine/Kg of dried coffee husk).

### Brix determination in colostrum and milk

All results for both experimental groups are presented in Table 1. Colostrum from sows supplemented with coffee husk showed significantly higher IgG concentrations, as estimated by Brix degrees, compared to the control group ( $t(18)=-2.258$ ,  $P=0.03$ ). This study revealed a slight but consistent increase of nearly 2.5 Brix degrees in the supplemented group, corresponding to approximately 2 to 3 mg/mL more IgG than in the colostrum of control sows. Given the moderate correlation between Brix degrees and IgG concentration ( $r=0.56$ ) as reported by Balzani (2016), Brix refractometry serves as an efficient and practical tool for on-farm estimation of immunoglobulin levels in colostrum. Similarly, milk samples collected 24 hours postpartum from supplemented sows also demonstrated significantly higher Brix values compared to the control group ( $t(18)=-2.929$ ,  $P=0.009$ ). The elevated IgG levels observed in both colostrum and milk from the supplemented group may be attributed to the biological effects of caffeine on mammary tissue.

Caffeine is known to promote mammary gland development by enhancing tissue sensitivity to mammatropic hormones and increasing intracellular cAMP levels, which in turn stimulates beneficial cellular activities. Additionally, caffeine inhibits phosphodiesterase, leading to cAMP accumulation in mammary epithelial cells, and influences pituitary hormone secretion by modulating neurotransmission (Li and Hacker, 1995). These mechanisms contribute to greater mammary gland development by stimulating expansion of the endoplasmic reticulum and growth of lobular structures, enabling enhanced fat globule accumulation and promoting lactogenesis (Segura *et al.*, 2020; Farmer *et al.*, 2006). Consequently, increased consumption of colostrum and milk combined with higher protein content in these secretions —is critical in the early life of piglets. It ensures greater intake of immunoglobulins, thereby enhancing passive immunity and reducing susceptibility to viral and bacterial infections (Superchi *et al.*, 2016).

### Lactational weight and preweaning mortality

Research has shown that caffeine is capable of crossing the placental barrier and can be detected in biological fluids such as colostrum and milk in pigs (Mazzoni *et al.*, 2012; Superchi *et al.*, 2013). In the present study, piglets born to sows supplemented with coffee husk alongside their regular diet showed no significant differences in birth weight compared to control piglets ( $t(221)=1.011$ ,  $P=0.31$ ;  $1.513 \pm 0.03$  kg *vs.*  $1.469 \pm 0.02$  kg,

**Table 1.** Brix degrees in colostrum and milk for both groups of sows.

Density (Brix degrees)	Colostrum (%) 6 h post-partum	Milk (%) 24 h post-partum	P-value
Control	$26.27 \pm 0.5$	$21.27 \pm 0.5$	0.030
Coffee by-products	$28.66 \pm 1.0$	$24.00 \pm 0.7$	0.009

for control and coffee husk groups, respectively). This outcome aligns with previous findings suggesting that caffeine administration during late gestation is safe, as the fetuses are already fully developed at that stage (Sánchez-Salcedo *et al.*, 2019; Superchi *et al.*, 2013).

However, at 21 days postpartum, piglets from the coffee husk-supplemented group exhibited significantly higher weaning weights ( $t(207) = -4.769$ ,  $P < 0.001$ ), averaging  $5.820 \pm 0.08$  kg compared to  $5.213 \pm 0.09$  kg in the control group. This outcome supports earlier research by Sánchez-Salcedo *et al.* (2019), who reported increased weaning weights in piglets following subcutaneous caffeine supplementation in sows during late gestation (Control:  $6.52 \pm 0.25$  kg *vs.* Caffeine:  $6.87 \pm 0.18$  kg,  $P < 0.05$ ). Conversely, Dearlove *et al.* (2018) found no significant effect on weaning weight with oral caffeine supplementation (6 g/day) starting on day 112 of gestation. The improved weight gain observed in this study likely results from caffeine's stimulatory effects, including enhanced colostrum and milk intake. Adequate colostrum consumption has been linked to increased daily weight gain during lactation and through weaning (Huting *et al.*, 2019; Quesnel and Farmer, 2019; Amatucci *et al.*, 2022). Caffeine is known to reduce lethargy by blocking adenosine receptors, thereby decreasing fatigue and increasing alertness. This heightened state of energy may encourage piglets to nurse more vigorously and frequently, promoting better nutrition (Dearlove *et al.*, 2018; Sanchez-Salcedo, 2019). Ensuring optimal development within the first 72 hours of life establishes a solid foundation for systematic feeding and adaptation, providing a developmental advantage over piglets not exposed to caffeine's stimulatory effects (Farmer *et al.*, 2006). Pre-weaning mortality is a key performance indicator in pig production systems, reflecting overall piglet and litter management, particularly in full-cycle farms. In this study, pre-weaning mortality was markedly lower in the caffeine-supplemented group (1 death; 0.97%) compared to the control group (13 deaths; 10.83%). Most piglet deaths were attributed to crushing by the sow. The significant reduction in mortality in the supplemented group is consistent with the positive effects of caffeine on neonatal vitality observed in previous studies (Dearlove *et al.*, 2018; Superchi *et al.*, 2016). Caffeine supplementation in sows, whether administered the day before induced farrowing (27 mg/kg) or for three days before natural farrowing (6 g/day), has been shown to reduce stillbirth rates and improve neonatal adaptation, including better thermoregulation, viability, and extended gestation by an average of 1.1 days (Dearlove *et al.*, 2015; Superchi *et al.*, 2013, 2016). In contrast, van Wettere *et al.* (2018) reported increased piglet mortality when sows received oral progesterone (0.4% w/v) and caffeine (2.4 g/kg feed) concurrently from days 111 to 113 of gestation. The authors suggested that the combined use of two uterine contractility inhibitors may have led to prolonged farrowing and increased mortality.

These findings support the hypothesis that improved nutrition and caffeine-induced stimulation enhance piglet reflexes and adaptive behavior, reducing risks such as asphyxia, dehydration, and crushing. Meanwhile, piglets from control sows may face early-life challenges linked to pain, stress, or inadequate care during and after farrowing, compromising their initial development and survival (Tummaruk and Kridtasak, 2012).

## CONCLUSIONS

This study demonstrates that supplementing pregnant sows with 150 g of coffee husk a natural source of caffeine —between days 110 and 112 of gestation effectively enhances piglet development without negatively impacting sow behavior or causing adverse outcomes such as abortion. The supplementation led to increased immunoglobulin G (IgG) concentrations in colostrum and milk, with levels 2 to 3 mg/mL higher than those observed in the control group. Additionally, piglets born to supplemented sows exhibited weight gains of up to 600 g more than those in the control group by weaning. Pre-weaning mortality at 21 days was also significantly reduced (0.97% in the coffee husk group *vs.* 10.83% in the control group).

These improvements in early piglet performance could enhance profitability in small-scale swine production systems and provide practical solutions to ongoing challenges in pig farming. However, to fully understand the role of caffeine administered through coffee husk, further pharmacokinetic and behavioral research is essential. Future studies should explore varying doses and administration durations to precisely determine the amount of caffeine transferred to piglets via colostrum and milk, as well as its potential effects on both sow and piglet physiology and development.

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