Research Article

Effects of Polyherbal Formulation As a Natural Replacer of Choline Chloride in Weaned Piglet Diets: Impacts on Performance and Blood Parameters

Rhuan Chaves[1], Diego Leal[2], Ygor de Paula[3], Andreia Vilas Boas[4], Ana Toledo[4], Sígfrid López-Ferrer[5], Vinicius Cantarelli[3], Cesar Garbossa[2*]

[1]Animalnutri Science and Technology, Lavras, Minas Gerais, Brazil; [2]University of São Paulo, Pirassununga, São Paulo, Brazil; [3]Federal University of Lavras, Minas Gerais, Brazil; [4]Vidara do Brasil Ltda, Jundiaí, São Paulo, Brazil; 5Vidara Life Ingredients SAU, Spain.

Abstract | Choline is a vitamin-like nutrient that plays a crucial role in several biological processes. Choline is typically added to pig diets in the form of choline chloride. However, the use of this synthetic choline has some disadvantages such as high capacity to absorb moisture, oxidative loss of vitamins in feed and its metabolism generates a compound that can taint animal-origin products. The aim of this study was to evaluate the effects of feeding diets containing natural choline (Kolin plus™ FC) as a replacement of choline chloride on performance and blood variables of weaned piglets. A total of 360 piglets with an average initial body weight of 6.33 ± 0.20 kg were utilized in a 41-day experiment. The experimental design followed a randomized blocks design, with three treatments and twelve replicates. Piglets were assigned to one of three treatments: Negative control (basal diet without choline chloride), Choline Chloride 60% (600 grams/ton supplementation), and Kolin Plus™ FC (20% choline chloride; Indukern, Brazil). Diets were formulated to meet or exceed nutritional requirements, and piglets had *ad libitum* access to feed and water throughout the experiment. The use of Kolin plus™ FC tended (p = 0.09) to improve the feed conversion ratio in the pre-starter II phase. No statistical differences were observed for the remaining performance variables. The mortality did not differ among treatments. There were no differences among treatments for any blood variable evaluated. In conclusion, the polyherbal formulation Kolin Plus™ FC successfully replaced choline chloride in weaned piglet diets without compromising the growth performance or health of nursery piglets.

Keywords | Choline; Polyherbal formulation; Nursery piglets; Growth performance; Health

Received | February 27, 2024; **Accepted** | May 16, 2024; **Published** | June 05, 2024
***Correspondence** | Cesar Garbossa,University of São Paulo, Pirassununga, São Paulo, Brazil; **Email:** cgarbossa@usp.br
Citation | Chaves R, Leal D, Paula YD, Boas AV, Toledo A, Lopez-Ferrer S, Cantarelli V, Garbossa C (2024). Effects of polyherbal formulation as a natural replacer of choline chloride in weaned piglet diets: impacts on performance and blood parameters. J. Anim. Health Prod. 12(3): 276-284.
DOI | http://dx.doi.org/10.17582/journal.jahp/2024/12.3.276.284
ISSN (Online) | 2308-2801

INTRODUCTION

Weaning is arguably one of the most stressful events in the life of the pig (Campbell et al., 2013). During this critical phase, piglets are exposed to a host of stressors, including removal from the sow, transportation, interaction with unacquainted pigs, and the transition from sow milk to a less digestible diet (Pluske et al., 1997). This can result in intestinal and immune system dysfunctions that lead to reduced pig health, feed intake and growth performance (Cabrera et al., 2013; Pié et al., 2004). Likewise, piglets at the time of weaning have reduced digestive enzymatic activity (Hedemann et al., 2006). Pancreatic lipases, pivotal enzymes in the process of lipid digestion and absorption, undergo marked alterations following weaning (Jensen et al., 1997). Consequently, the capacity for digesting and absorbing dietary lipids in weaned piglets experiences a significant decline (Qiu et al., 2021).

In recent years, in order to mitigate the biological stress of weaning, improve nutrient utilization and growth performance, several nutritional strategies have been evaluated (Campbell et al., 2013; Jayaraman & Nyachoti, 2017; Kim et al., 2012). These includes, but are not limited to, dietary inclusion of probiotics (Azizi et al., 2022), phytochemicals (Xu et al., 2021), dietary amino acids (Liao, 2021), organic and inorganic acids (Liu et al., 2018), acidifiers, bacteriophages (Rahman et al., 2022) and exogenous enzymes (Moita & Kim, 2022). Micronutrients (i.e., trace minerals and vitamins) are critical factors affecting health, growth and development in pigs (Matte & Audet, 2020).

Choline (trimethyl-beta-hydroxyethylammonium) is a vital water-soluble nutrient that plays multifaceted roles in neurotransmission, membrane integrity, and methylation pathways (Li et al., 2015; Zeisel, 1981), exerting influence over various biological processes (Corbin and Zeisel, 2012). Notably, choline performs a crucial role in lipid regulation; acting as a methyl group donor and a precursor of phosphatidylcholine, it contributes to the assembly of very low-density lipoproteins (VLDLs) in the liver (Musso et al., 2009). Additionally, as a significant component of the mitochondrial membrane, choline plays a pivotal role in averting oxidative damage resulting from mitochondrial dysfunction (Corbin and Zeisel, 2012).

In poultry, a number of studies have demonstrated that supplemental choline exerted beneficial effects on growth performance. Broilers supplemented with choline had higher feed intake, improved feed conversion ratio and weight gain (Farina et al., 2017). Likewise, Selvam et al. (2018) reported that growth performance and feed efficiency were improved in broilers supplemented with choline. In pigs, the studies on evaluating choline supplementation are more limited. Choline supplementation has been demonstrated to enhance growth performance and mitigate gut inflammation in weaned piglets by modulating gut microbiota and lipid metabolism (Qiu et al., 2021). Moreover, dietary choline supplementation has been linked to improved birth weight, enhanced uniformity among neonatal piglets, and enhanced litter performance during lactation, likely attributable to its enhanced antioxidant capacity and metabolic status (Zhong et al., 2022). Additionally, dietary choline supplementation has been shown to enhance fat deposition by promoting lipogenesis and reducing lipolysis in intrauterine growth retardation piglets (Li et al., 2015). Choline is typically added to pig diets in the form of choline chloride, however, the use of choline chloride has some drawbacks, including high hygroscopicity, acceleration of oxidative loss of vitamins, its corrosive nature is a practical concern in processing units and feed mills, and since less than half of the choline chloride is absorbed, the remaining is converted to trimethylamine (TMA) in the gastro intestinal tract (Selvam et al., 2018; Zeisel et al., 1989), Trimethylamine is a compound that has an unpleasant odor and can taint animal-origin food products (Selvam et al., 2018).

Currently, there is a growing interest in achieving more sustainable pig production, leading to increased exploration of phytogenics (Rahman et al., 2022). In this context, Kolin Plus™ FC stands out as a polyherbal formulation composed of a blend of *Acacia nilotica* and *Curcuma longa* (Selvam et al., 2018). Extracts from these plants have been found to possess hepatoprotective, antioxidant, and lipotropic properties (Kannan et al., 2013; Yarru et al., 2009). Kolin Plus™ FC has shown promising results in replacing synthetic choline (choline chloride 60%) in broiler diets, leading to improved growth performance and feed efficiency (Selvam et al., 2018). However, there is limited research on the use of natural choline sources as replacements for choline chloride in pigs.

Based on the foregoing, the objective of this study was to evaluate the effects of feeding weaned piglets on diets containing choline from a polyherbal source (Kolin plus™ FC) as a replacement for choline chloride 60% on their performance and mortality.

MATERIALS AND METHODS

The animal study was conducted in accordance with the ethical guidelines and regulations established by the Animal Nutrition Animal Care and Use Committee, with approval granted under CEUA protocol number 008/22.

Animals, housing, experimental design, and diets

The experiment was carried in the nursery unit of the Animalnutri & Auma Experimental Center of Swine (18.5962°S, 46.5150°W, Patos de Minas, Minas Gerais, Brazil). A total of 360 crossbred piglets (DB90 [Large white x Landrace] x LQ 1250 [Duroc]; DB Genética Suína, Patos de Minas, Brazil) with an initial average body weight (BW) of 6.33 ± 0.20 kg and 23 ± 1 days of age were allocated in slatted pens equipped with semi-automatic feeders and drinking nipple. The temperature was controlled manually according to the age of the animals by opening or closing the windows. Temperature thermometers were used to indicate when the windows should be closed or opened. The room temperature was recorded daily. The experimental design was a randomized blocks design, with three treatments and twelve replicates (six male and six female). The pen (10 piglets) was considered the experimental unit. The initial weight was used as a block factor. The experiment lasted 41 days and was divided in four periods: pre-starter I (0 to 7 days of the trial), pre-starter II (7 to 14 days of the trial), starter I (14 to 27 days of the trial)

and starter II (27 to 41 days of the trial). The piglets were allotted to one of three treatments as follows: Negative control (NC, n = 120, basal diet without choline chloride); Choline Chloride 60% (CC60%, n = 120, chloride supplementation – 600 grams/ton) serving as positive control and Kolin Plus™ FC (n = 120, corresponding to 20% of choline chloride; Indukern, Brazil). The experimental diets were formulated to meet or exceed the nutritional requirements of each phase, according to the Brazilian Poultry and Swine Tables (Rostagno et al., 2017) (Table 1). Piglets had *ad libitum* access to feed (mash) and water until the end of the experiment.

Growth Performance and mortality

The piglets were weighed at day 0, 7, 14, 27 and 41 of the experimental periods and the experimental diets were changed on the same days. The feed provided and refusals were evaluated daily. Thus, the average daily gain (ADG), the average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated weekly in the aforementioned experimental periods. When a piglet died in the pen, the dead piglet was weighed as well as the litter mates, and the feed provided to the pen. The medication rate was calculated as the percentage of individual piglets treated with injectable antibiotics. Mortality was calculated as the percentage of piglets that died in each dietary treatment during the experimental period. Apathy was diagnosed by direct observation. For this study, piglets were considered to exhibit signs of apathy when they remained in lateral or sternal recumbency for extended periods, showed no interest in consuming feed, and did not have a fever.

Blood collection and analysis

At the end of the experiment, blood samples were collected (10 replicates per treatment; piglets with the closest weight to the pen average) via venipuncture. Blood was allowed to clot at room temperature and was stored at -20 until analysis. The serum levels of cholesterol and total triglycerides were determined using the enzymatic Trinder method. The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was determined by the U.V Kinetic method. All samples were analyzed in the AU480 Chemistry Analyzer equipment (Beckman Coulter, Brea, USA). All blood analyses were conducted following the methodology proposed by Stricker-Krongrad et al. (2016).

Statistical Analysis

All residues were tested for normality and homogeneity using the Shapiro-Wilk test. When variables did not follow a normal distribution the PROC RANK (SAS INSTITUTE INC, 2009) procedure was used to produce a transformed variable. The data was analyzed as a randomized complete block design using the PROC GLIMMIX procedure in SAS 9.3 (SAS Inst. Inc., Cary, NC). The pen was used as the experimental unit. Dietary treatments were considered as fixed effects and blocks as random effects. All data were described as LSMEANS. The medication and mortality rates were analyzed using the chi-square test by the GMOD procedure of SAS. The means were compared by Tukey test. Differences were considered significant if $p < 0.05$, with a trend toward significance at $0.05 \leq p \leq 0.10$.

RESULTS

Growth performance

The results of growth performance were displayed in Table 2. The use of Kolin plus™ FC only tended to improve (p = 0.09) FCR in the pre-starter II phase. In the starter II phase, the Kolin plus™ FC group had numerically better (p = 0.45) FCR compared to Choline Chloride 60%. In the pre-starter II and starter II phases the Kolin plus™ FC treatment had numerically greater (p = 0.12 and p = 0.35, respectively) ADG values.

Medication and mortality incidences

The incidence of medication is described in Table 3. In the pre-starter I phase, no piglets were medicated. In the pre-starter II phase, one piglet from the Kolin plus™ FC treatment was medicated for arthritis. In the starter I phase, a total of nine piglets were medicated. Of these, one piglet from the negative control treatment (due to apathy); four from the choline chloride 60% treatment (two for diarrhea, one due to apathy and one for arthritis) and four piglets from the Kolin plus™ FC treatment (two for diarrhea, one for encephalitis and one due to apathy). Regarding mortality, there were no deaths in the pre-starter II and starter II phases. In the pre-starter I phase, one animal from the Kolin plus™ FC treatment died, showing respiratory symptoms. In the starter I phase, one animal from the Kolin plus™ FC treatment died, with arthritis being the only alteration observed. In the same period, two piglets from the Kolin plus™ FC treatment were removed from the experiment. These piglets had already been medicated, notwithstanding, none of them showed signs of improvement.

Blood Analysis

The results of blood analysis are described in Table 4. No statistical difference was observed for any blood parameter evaluated. The values of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were within normal range for piglets from all treatment groups.

DISCUSSION

Although no statistical differences were observed for most of the performance variables evaluated, a pivotal finding of this study is the successful replacement of choline chlo

Table 1: Composition of the experimental diets.

Ingredients (%)	Pre-starter I	Pre-starter II	Starter I	Starter II
Corn 7.88% CP	43.6	48.8	55.7	58.5
Soybean meal 46% CP	15.7	19.7	25.0	32.0
Spray-dried plasma	5.0	3.0	1.5	-
Dried milk	9.0	6.0	-	-
Dried whey	20.0	12.5	7.5	-
Soybean oil	3.0	2.64	2.84	2.77
Sugar	-	2.5	2.5	2.5
Vitamin premix[1]	0.05	0.05	0.05	0.05
Micromineral premix[2]	0.1	0.1	0.1	0.1
Phytase 10.000FTU	0.01	0.008	0.008	0.008
Novicid 18 CA[3]	0.5	0.4	0.3	0.25
Adimix Precision[4]	0.2	0.15	0.1	0.1
Antioxidant	0.02	0.02	0.02	0.02
Flavoring	0.05	0.05	-	0.05
Dicalcium phosphate 18.5% P	0.428	0.839	1.093	0.935
Limestone	0.658	1.22	1.143	0.877
Salt	-	0.118	0.30	0.5
Copper sulfate	0.03	0.05	0.05	0.05
Zinc oxide 80%	0.3	0.24	0.18	0.15
L-Lysine-HCL	0.444	0.546	0.59	0.437
DL-methionine	0.239	0.253	0.23	0.174
L-treonine	0.244	0.28	0.29	0.189
L-tryptophan	0.059	0.06	0.059	0.026
L-valine	0.085	0.135	0.156	-
Inert	0.283	0.341	0.291	0.314
Total	100.	100	100	100
Calculated levels (%)				
ME (kcal/kg)	3459.5	3400.0	3375.0	3350.0
Fat	5.30	5.10	5.55	5.62
Crude protein	20.6	19.6	18.8	19.7
SID Lysine	1.52	1.45	1.35	1.25
SID Methionine	0.52	0.52	0.47	0.43
SID Met + Cys	0.85	0.81	0.75	0.71
SID Threonine	1.02	0.97	0.90	0.81
SID Tryptophan	0.29	0.28	0.26	0.24
SID Arginine	1.01	1.01	1.06	1.20
SID Valine	1.05	1.00	0.93	0.81
SID Isoleucine)	0.78	0.73	0.68	0.74
SID Leucine	1.68	1.55	1.43	1.50
SID Histidine	0.52	0.48	0.44	0.47
SID Phenyalanine	0.89	0.84	0.80	0.87
Crude fibre	1.53	1.81	2.18	2.57
Lactose	17.6	11.15	5.25	-
Total Calcium	0.88	1.07	0.97	0.79
Available Phosphorus	0.55	0.53	0.48	0.39

Sodium	0.32	0.25	0.23	0.22
Copper (mg/kg)	98.2	147.3	146.4	146.4
Zinc (mg/kg)	2565.8	2076.8	1588.1	1343.3
Choline (mg/kg)	1230.0	1178.7	1177.8	1238.1

[1]The vitamin premix provided the following quantities of vitamins per kg of complete diet: 30.000.000 IU vitamin A, 6.000.000 IU vitamin D3, 180.00 IU vitamin E, 6.700 mg vitamin K3, 5.250 mg, 11.88 g vitamin B2, 47.5 g pantothenic acid, 6.880 vitamin B6, 68.750 mcg vitamin B12, 75 g nicotinic acid, 6.880 mg folic acid, 810 mg biotin.
[2]The micro mineral premix provided the following quantities of micro minerals per kg of complete diet: 80 g iron, 15 g copper, 41.3 g manganese, 91.2 g zinc, 330 g cobalt, 1.209 mg iodine, 351 mg selenium.
[3]Acidifier.
[4]Encapsulated sodium butyrate.

Table 2: Effect of the experimental diets on piglet performance in each period of evaluation

Variables	**Treatments**			**SEM**	**P**
	NC	**CC60%**	**Kolin plus™ FC**		
Initial BW, kg	6.33	6.33	6.33	0.26	0.72
0 to 7 days					
BW at 7 days, kg	7.69	7.77	7.72	0.31	0.52
ADFI, kg	0.31	0.32	0.32	0.01	0.23
ADG, kg	0.19	0.2	0.19	0.01	0.53
FCR	1.59	1.58	1.64	0.05	0.45
7 to 14 days					
BW at 14 days, kg	10.0	10.0	10.1	0.39	0.42
ADFI, kg	0.54	0.55	0.55	0.01	0.75
ADG, kg	0.33	0.32	0.35	0.01	0.12
FCR	1.65	1.7	1.58	0.04	0.09
14 to 27 days					
BW at 27 days, kg	16.6	16.5	16.7	0.59	0.43
ADFI, kg	0.77	0.75	0.78	0.02	0.26
ADG, kg	0.50	0.50	0.5	0.01	0.76
FCR	1.52	1.51	1.53	0.02	0.71
27 to 41 days					
BW at 41 days, kg	26.3	25.9	26.7	0.71	0.13
ADFI, kg	1.14	1.11	1.14	0.04	0.38
ADG, kg	0.69	0.67	0.7	0.01	0.35
FCR	1.64	1.66	1.61	0.02	0.58
0 to 41 days					
ADFI, kg	0.78	0.77	0.78	0.02	0.30
ADG, kg	0.48	0.47	0.49	0.01	0.19
FCR	1.6	1.6	1.58	0.02	0.70

NC: negative control; CC60%: choline chloride 60%: BW: bodyweight; ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio; SEM: standard error of the mean.

ride in weaned piglet diets with the polyherbal formulation Kollin Plus™ FC, without compromising growth performance and nursery piglet health. This aligns with findings by Selvam et al. (2018), who demonstrated that this polyherbal formulation effectively replaced the function of 1 kg/ton of synthetic choline (choline chloride 60%) at a 400 g/ton inclusion rate in broiler diets. Moreover, in their study, the replacement of synthetic choline by the natural choline resulted in improved feed efficiency and growth performance. In the present study, the use of choline from a polyherbal source only tended to affect FCR in the pre-starter II phase. Only numerical differences were

Table 3: Effect of the experimental diets on the incidence of medication in each experimental period

Period	Treatments			Total
	NC	CC60%	Kolin plus™ FC	
Pre-starter I	0	0	0	0
Pre-starter II	0	0	1	1
Starter I	1	4	4	9
Starter II	0	1	0	1
Total	1	5	5	11

NC: Negative control; CC60%; Choline Chloride 60%

Table 4: Effects of the experimental diets on serum metabolites (Mean).

Variables	Treatments			SEM	P
	NC	CC60%	Kolin plus™ FC		
Cholesterol (mg/dl)	70.3	66.0	69.5	2.44	0.34
Triglycerides (mg/dl)	33.3	32.3	34.1	1.89	0.87
AST (U/L)	39.3	39.7	37.2	2.13	0.84
ALT (U/L)	39.3	40.8	41.8	1.74	0.76

NC: Negative control; CC60% = Choline Chloride 60%; SEM: standard error of the mean; AST: aspartate aminotrans-ferase; ALT: alanine aminotransferase.

observed among treatments for the remaining performance variables, including the negative control group (diet without choline inclusion). This trend contrasts with the findings of Qiu et al. (2021), who reported significant improvements in growth performance metrics such as average daily gain (ADG), feed conversion ratio (FCR), and overall body weight gain in weaned piglets with choline chloride supplementation compared to diets lacking choline. Notably, previous research suggests that choline supplementation may not significantly impact pig performance when dietary methionine and vitamin B content is sufficient (Siljander-Rasi et al., 2003), as observed in the present study. Further research is warranted to investigate the effects of supplemental choline in methionine and vitamin deficient diets on growth performance of weaned piglets.

The incidence of medication and mortality rates observed in the present experiment demonstrates that the use of choline from a polyherbal source had no deleterious effect on piglet health. Further, only two piglets from the Kolin plus™ FC treatment died during the experimental period, one had respiratory symptoms and the other one had arthritis. Aside these alterations, none of the piglets that died had other signs indicating metabolic alterations or intoxication. Only one piglet from the Kolin Plus™ FC treatment was medicated due to apathy. The absence of deleterious effects is corroborated by the results of the blood analysis. Indeed, there was no difference in the levels of AST and ALT among treatments, indicating normal liver function. The absence of deleterious effects of Kolin Plus™ FC is not surprising given the fact that it is a polyherbal composed by *A. nilotica* and *C. longa*, a rich source of polyphenols and curcuminoids, which have proven hepatoprotective effects (Kannan et al., 2013). Further, it has been demonstrated that the supplementation of *C. longa* extracts had a beneficial impact on transmethylation pathways and osmotic regulation by increasing the liver betaine content, which plays a role in liver lipid metabolism (Tranchida et al., 2015).

Research efforts have consistently shown that choline supplementation improves lipid metabolism by reducing circulating levels of free fatty acids and triglycerides (Li et al., 2018; Qiu et al., 2021). However, in our study, supplementing weaned piglet diets with choline, regardless of its source (natural or synthetic), did not affect cholesterol and triglyceride levels. Moreover, these levels remained similar between piglets in the negative control group and those in the choline-supplemented groups. Li et al. (2018) suggested that choline oxidase activity in pig livers is relatively lower compared to that in broiler chickens, potentially delaying the manifestation of significant effects of choline or its metabolites in pigs. This might explain the absence of observable effects on lipid metabolism in our study. Nevertheless, it is important to note that, similar to AST and ALT levels, cholesterol and triglyceride levels in piglet blood across all treatment groups fell within the normal range.

A reduced digestive enzymatic activity of young piglets is important factor that contributes to the occurrence of diarrhea in the nursery phase (Qiu et al., 2021). For instance, lipase activity is dramatic reduced after weaning (Jensen et al., 1997). The supplementation of Choline has

been shown to efficiently emulsify dietary fat, accelerating lipid metabolism in the small intestine (Leermakers et al., 2015). It should be pointed out that piglets were kept under good sanitary conditions and no challenge was impose. The low incidence of medication and mortality observed in this study corroborates this assumption. Future trials should evaluate the use of natural choline in diets of challenged-piglets.

CONCLUSIONS

The supplementation of Kolin Plus™ FC in replacement of choline chloride 60% proved to be feasible and safe, with no statistical difference between the two treatments for the majority of the performance variables evaluated. Additionally, the supplementation of nursery piglet diets with Kolin Plus™ FC in replacement of choline chloride 60% tended to improve FCR in the pre-starter II phase. Further, the serum determination of AST and ALT demonstrated that no liver injury occurred in piglets supplemented with Kollin Plus™ FC. Although choline is involved in lipid metabolism, no effect of choline, regardless of source was observed for serum cholesterol and triglycerides. Further studies are needed to evaluate the use of Kolin Plus™ FC in replacement of synthetic choline in nursery piglet diets during a more extended period of time and under challenging conditions.

ACKNOWLEDGEMENTS

The authors thank National Council for Scientific and Technological Development (CNPq) for the fellowships: 303750/2021-9 and 312659/2019-9.

FUNDING

This study was funded by Natural Remedies Private Limited, Bangalore, India.

AVAILABILITY OF DATA AND MATERIALS

The data sets and materials are available from the corresponding author upon reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The animal study was approved by Animalnutri animal care and use committee (CEUA protocol number: 008/22).

CONFLICT OF INTERESTS

The authors A.V.B., A.T., S.L.F. are affiliated with the company that distributes the technology, and it may influence the way in which the technology is presented and discussed in this publication. However, we want to assure our readers that we have taken measures to minimize any potential biases. We have followed rigorous scientific methods and have reported our findings in an objective and transparent manner. We have also enlisted the help of independent reviewers to assess the quality and validity of our work.

NOVELTY STATEMENT

This study presents novel insights into the use of natural choline sources in piglet nutrition. Traditionally, choline chloride is used in pig diets despite its drawbacks, including high moisture absorption, oxidative loss of vitamins in feed, and the generation of undesirable metabolites. This research evaluates the effects of Kolin Plus™ FC, a natural choline alternative, on the performance and blood variables of weaned piglets. The findings indicate that Kolin Plus™ FC can effectively replace choline chloride without negatively impacting growth performance or health, suggesting a viable and potentially superior alternative for piglet diets. This is the first comprehensive evaluation of Kolin Plus™ FC in this context, highlighting its potential to improve feed conversion ratios and mitigate the disadvantages associated with synthetic choline.

AUTHORS CONTRIBUTIONS

Conceptualization, A.V.B., A.T., S.L.F., R.C., V.C. and C.G..; methodology, A.V.B., A.T., S.L.F., R.C., R.C., V.C., and C.G..; formal analysis, R.C.; investigation, R.C., D.L., Y.P.; resources, R.C. and V.C.; data curation, R.C. and Y.P.; writing-original draft preparation, R.C., D.L., and Y.P.; writing-review and editing, A.V.B., A.T., S.L.F., R.C., V.C., and C.G.; supervision, R.C., V.C., and C.G.; project administration, R.C.; funding acquisition, R.C. and V.C. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Azizi A. F. N., Uemura R., Omori M., Sueyoshi M., Yasuda M. (2022). Effects of Probiotics on Growth and Immunity of Piglets. Animals., 12(14): 1–8. https://doi.org/10.3390/ani12141786

Cabrera R. A., Usry J. L., Arrellano C., Nogueira E. T., Kutschenko M., Moeser A. J., Odle J. (2013). Effects of creep feeding and supplemental glutamine or glutamine plus glutamate (Aminogut) on pre- and post-weaning growth

performance and intestinal health of piglets. J. Anim. Sci. Biotechnol., 4(1): 1. https://doi.org/10.1186/2049-1891-4-29

Campbell J. M., Crenshaw J. D., Polo J. (2013). The biological stress of early weaned piglets. J. Anim. Sci. Biotechnol., 4(1): 2–5. https://doi.org/10.1186/2049-1891-4-19

Corbin K. D., Zeisel S. H. (2012). Choline metabolism provides novel insights into nonalcoholic fatty liver disease and its progression. Curr. Opin. Gastroenterol., 28(2): 159–165. https://doi.org/10.1097/MOG.0b013e32834e7b4b

Farina G., Kessler A. de M., Ebling P. D., Marx F. R., César R., Ribeiro A. M. L. (2017). Performance of Broilers Fed Different Dietary Choline Sources and Levels. Ciência Anim. Brasil., 18(0): 1–14. https://doi.org/10.1590/1089-6891v18e-37633

Hedemann M. S., Eskildsen M., Lærke H. N., Pedersen C., Lindberg J. E., Laurinen P., Knudsen K. E. B. (2006). Intestinal morphology and enzymatic activity in newly weaned pigs fed contrasting fiber concentrations and fiber properties1. J. Anim. Sci., 84(6): 1375–1386. https://doi.org/10.2527/2006.8461375x

Jayaraman B., Nyachoti C. M. (2017). Husbandry practices and gut health outcomes in weaned piglets: A review. Anim. Nutrit., 3(3): 205–211. https://doi.org/10.1016/j.aninu.2017.06.002

Jensen M. S., Jensen S. K., Jakobsen K. (1997). Development of Digestive Enzymes in Pigs with Emphasis on Lipolytic Activity in the Stomach and Pancreas. J. Anim. Sci., 75(2): 437–445. https://doi.org/10.2527/1997.752437x

Kannan N., Sakthivel K. M., Guruvayoorappan C. (2013). Protective effect of Acacia nilotica (L.) against acetaminophen-induced Hepatocellular Damage in Wistar rats. Adv. Pharmacolog. Sci., 2013. https://doi.org/10.1155/2013/987692

Kim J. C., Hansen C. F., Mullan B. P., Pluske J. R. (2012). Nutrition and pathology of weaner pigs: Nutritional strategies to support barrier function in the gastrointestinal tract. Animal Feed Sci. Technol., 173(1–2): 3–16. https://doi.org/10.1016/j.anifeedsci.2011.12.022

Leermakers E. T. M., Moreira E. M., Kiefte-de Jong J. C., Darweesh S. K. L., Visser T., Voortman T., Bautista P. K., Chowdhury R., Gorman D., Bramer W. M., Felix J. F.,nFranco O. H. (2015). Effects of choline on health across the life course: A systematic review. Nutrit. Rev., 73(8): 500–522. https://doi.org/10.1093/nutrit/nuv010

Li B., Li W., Ahmad H., Zhang L., Wang C., Wang T. (2015). Effects of choline on meat quality and intramuscular fat in intrauterine growth retardation pigs. PLoS ONE., 10(6): 1–13. https://doi.org/10.1371/journal.pone.0129109

Li W., Li B., Lv J., Li D., Zhang L., Wang T. (2018). Choline supplementation improves the lipid metabolism of intrauterine-growth-restricted pigs. Asian-Australasian J. Anim. Sci., 31(5): 686–695. https://doi.org/10.5713/ajas.15.0810

Liao S. F. (2021). Invited review: Maintain or improve piglet gut health around weanling: The fundamental effects of dietary amino acids. Anim., 11(4): 1–16. https://doi.org/10.3390/ani11041110

Liu Y., Espinosa C. D., Abelilla J. J., Casas G. A., Lagos L. V., Lee S. A., Kwon W. B., Mathai J. K., Navarro D. M. D. L., Jaworski N. W., Stein H. H. (2018). Non-antibiotic feed additives in diets for pigs: A review. Anim. Nutri., 4(2): 113–125. https://doi.org/10.1016/j.aninu.2018.01.007

Matte J. J., Audet I. (2020). Maternal perinatal transfer of vitamins and trace elements to piglets. Animal., 14(1): 31–38. https://doi.org/10.1017/S175173111900140X

Moita V. H. C., Kim S. W. (2022). Nutritional and Functional Roles of Phytase and Xylanase Enhancing the Intestinal Health and Growth of Nursery Pigs and Broiler Chickens. Animals., 12(23). https://doi.org/10.3390/ani12233322

Musso G., Gambino R., Cassader M. (2009). Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD). Progress Lipid Res., 48(1): 1–26. https://doi.org/10.1016/j.plipres.2008.08.001

Pié S., Lallès, J. P., Blazy, F., Laffitte, J., Sève, B., Oswald, I. P. (2004). Weaning Is Associated with an Upregulation of Expression of Inflamatory Cytokines in the Intestine of Piglets. J. Nutrit., 134(3), 641–647. https://doi.org/10.1093/jn/134.3.641

Pluske J. R., Hampson D. J., Williams I. H. (1997). Factors influencing the structure and function of the small intestine in the weaned pig: A review. Livest. Prod. Sci., 51(1–3): 215–236. https://doi.org/10.1016/S0301-6226(97)00057-2

Qiu Y., Liu S., Hou L., Li K., Wang L., Gao K., Yang X., Jiang Z. (2021). Supplemental Choline Modulates Growth Performance and Gut Inflammation by Altering the Gut Microbiota and Lipid Metabolism in Weaned Piglets. J. Nutrit., 151(1): 20–29. https://doi.org/10.1093/jn/nxaa331

Rahman M. R. T., Fliss I., Biron E. (2022). Insights in the Development and Uses of Alternatives to Antibiotic Growth Promoters in Poultry and Swine Production. Antibiotics, 11(6): 1–29. https://doi.org/10.3390/antibiotics11060766

Rostagno H. S., Albino L. F. T., Donzele J. L., Gomes P. C., de Oliveira R. F., Lopes D. C., Ferreira A. S., Barreto S. L. T., Euclides R. (2017). Tabelas Brasileiras Para Aves e Suínos: Composição de Alimentos e Exigências Nutricionais. In Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais (4rd ed.). UFV.

Statistical analysis system. (2011) Version 9.3. Cary: SAS Institute.

Selvam R., Saravanakumar M., Suresh S., Chandrasekeran C. V., Prashanth D. (2018). Evaluation of polyherbal formulation and synthetic choline chloride on choline deficiency model in broilers: Implications on zootechnical parameters, serum biochemistry and liver histopathology. Asian-Australasian J. Anim. Sci., 31(11): 1795–1806. https://doi.org/10.5713/ajas.18.0018

Siljander-Rasi H., Peuranen S., Tiihonen K., Virtanen E., Kettunen H., Alaviuhkola T., Simmins P. H. (2003). Effect of equi-molar dietary betaine and choline addition on performance, carcass quality and physiological parameters of pigs. Anim. Sci., 76(1): 55–62. https://doi.org/10.1017/s1357729800053315

Stricker-Krongrad A., Shoemake C.R., Pereira M.E., Gad S.C., Brocksmith D., Bouchard G.F. (2016). Miniature Swine Breeds in Toxicology and Drug Safety Assessments: What to Expect during Clinical and Pathology Evaluations. Toxicolog. Pathol., 44(3): 421-427. https://doi.org/10.1177/0192623315613337

Tranchida F., Shintu L., Rakotoniaina Z., Tchiakpe L., Deyris V., Hiol A., Caldarelli S. (2015). Metabolomic and lipidomic analysis of serum samples following Curcuma longa extract supplementation in high-fructose and saturated fat fed rats. PLoS ONE., 10(8): 1–19. https://doi.org/10.1371/journal.pone.0135948

Xu B., Qin W., Xu Y., Yang W., Chen Y., Huang J., Zhao J., Ma L. (2021). Dietary Quercetin Supplementation Attenuates Diarrhea and Intestinal Damage by Regulating Gut Microbiota in Weanling Piglets. Oxidat. Med. Cellul. Longevit., 2021. https://doi.org/10.1155/2021/6221012

Yarru L. P., Settivari R. S., Gowda N. K. S., Antoniou E., Ledoux D. R., Rottinghaus G. E. (2009). Effects of turmeric (Curcuma longa) on the expression of hepatic genes associated with biotransformation, antioxidant, and immune systems in broiler chicks fed aflatoxin. Poult. Sci., 88(12): 2620–2627. https://doi.org/10.3382/ps.2009-00204

Zeisel S. H. (1981). Dietary choline: biochemistry, physiology, and pharmacology. Annual Rev. Nutr., 1: 95–121. https://doi.org/10.1146/annurev.nu.01.070181.000523

Zeisel S. H., DaCosta K. A., Youssef M., Hensey S. (1989). Conversion of dietary choline to trimethylamine and dimethylamine in rats: Dose-response relationship. J. Nutr., 119(5): 800–804. https://doi.org/10.1093/jn/119.5.800

Zhong W., Hu L., Zhao Y., Li Z., Zhuo Y., Jiang X., Li J., Zhao X., Che L., Feng B., Lin Y., Xu S., Fang Z., Wu D. (2022). Effects of Dietary Choline Levels During Pregnancy on Reproductive Performance, Plasma Metabolome and Gut Microbiota of Sows. Front. Vet. Sci., 8. https://doi.org/10.3389/fvets.2021.771228