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Effect of vitamin C and iron supplementation in pregnant hyper-prolific sows

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HIGHLIGHTS

- Vitamin C and vitamin C+Iron supplementations overcome low antepartum hemoglobin.
- Both supplementations increased the birth weight of piglets only for parity 1–2.
- We found no clear advantage of vitamin C+Iron over vitamin C.
- Vitamin C alone would be sufficient to achieve beneficial effects.

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ABSTRACT

Sow nutrition plays a crucial role impact on fetal and neonates' development, lactation and lifetime production of piglets. In this field study, our aim was to evaluate the impact of two feed supplements, namely vitamin C (VC group) and a combination of vitamin C and ferrous sulfate (VC+Fe group), administered during the last trimester of pregnancy, on sow's blood hemoglobin (Hb) concentration and individual birth weight of piglets. The sows came from hyper-prolific genetic lines (Topigs Norsvin Ninety pregnant sows were randomly divided into three groups: 31, 29 and 30 individuals for the C (control), VC and VC+Fe groups, respectively. We measured individual Hb concentration I (Hb I) on day 76 of pregnancy for all groups, prior to the start of supplementation in the VC and VC+Fe groups. A second measurement (Hb II; antepartum hemoglobin) occurred 2–4 days before the estimated farrowing date for each sow. We recorded the individual birth weight (kg) of 376 piglets in the C group, 403 piglets in the VC group, and 392 piglets in the VC+Fe group. Both the VC and VC+Fe groups showed improvement in Hb II ($P = 0.005$), improving their hematological status; however, there was no significant advantage of VC+Fe over VC in terms of Hb II. The effect of group on individual birth weight of piglets varied depending on the parity of the mother ($P = 0.001$). The supplementation with both VC and VC+Fe demonstrated positive effects on the birth weight of piglets, but only in sows with parity 1 and 2 ($P < 0.001$). These results suggest that additional iron supplementation did not confer significant benefits beyond what was already provided in the pregnancy feed. Therefore, the inclusion of vitamin C supplementation alone appears to be sufficient.

1. Introduction

Over the past 30 years, there has been an increase in the iron (Fe) requirements for pregnant sows (Buffler et al., 2017). This coincides with the rise in litter size of hyper-prolific sows, reaching 18–20 piglets, as well as longer farrowing periods, smaller average piglet birth weights, and reduced individual vitality (Kemp et al., 2018). For the last four decades, the recommended Fe supplementation for pregnant sows has

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been 80–100 mg/kg of dry feed (National Research Council, NRC, 1979, 2012). However, this supplementation strategy falls short of meeting the iron requirement for these hyper-prolific sows (Bhattarai et al., 2019). Currently, gestation feed for sows includes Ferrous Sulphate Monohydrate (FeSO4H2O) as a source of iron supplementation, ranging from 70 to 120 mg/kg. The absorption of iron in animals is greatly hindered by its intestinal absorption. The main source of iron is derived from vegetables (non-heme iron) in the form of ferric Fe3+. To facilitate its absorption, it needs to be reduced to its ferrous state (Fe2+) to pass through the intestinal mucosal membrane (García Rosolen et al., 2010; Anderson and Frazer, 2017).

The enzyme duodenal cytochrome B (DCYTB) aids in the reduction of ferric iron through electron transfer from intracellular ascorbate. This mechanism highlights the role of vitamin C in enhancing iron absorption (Monsen, 1988; Suttle, 2010). Additionally, vitamin C contributes to the formation of low-molecular-weight iron chelates, further increasing iron absorption (Gulec et al., 2014). Vitamin C has also been associated with erythropoiesis and the storage and mobilization of ferritin, a protein that stores iron (Tarng, 2007; Sommano et al., 2011). However, specific indications regarding the dosage and necessity of ascorbic acid in pig diets are still unclear.

Although standard diets for pregnant sows already contain iron, the addition of a vitamin C supplement may enhance its absorption. Furthermore, considering the increased iron requirements due to high prolificacy, pregnant sows might benefit from additional iron supplementation supported by a vitamin C supplement. Therefore, the objective of this field study was to evaluate the effect of two feed supplements, vitamin C (VC group) and vitamin C + ferrous sulphate (VC + Fe group), administered during the last third of gestation, on sow blood hemoglobin (Hb) concentration and individual birth weight of piglets.

2. Material and methods

2.1. Ethics statement

This study complied with ARRIVE guidelines (Kilkeny et al., 2010), and we conducted it according to the Directive 2010/63/EU of the European Parliament and the Council of 22 September (2010) on the protection of animals used for scientific purposes. We cared for and used the animals in this study in accordance with the Spanish Policy for Pig Protection (RD, 1135/2002), which meets the European Directive on this subject (DOUE-L-2009-80287, 2009). The experiments complied with the precepts of animal welfare, as approved by the Committee of Ethics in Animal Experimentation of Universidad de Zaragoza (protocol no. PI46/21).

2.2. Animals

All the animals belonged to a commercial farm in Northeastern Spain, whose target was piglet production. Sows were from Topigs Norsvin line (87 % farrowing rate, 15 total piglets/litter, 13.1 live-born piglets/litter and 1.5 stillborn piglets/litter, 23 days of lactation, 11.9 weaned piglets/litter).

2.3. Experimental design

We enrolled a total of 90 pregnant sows from the same weekly farrowing batch in our study. We excluded sows with dystocic parturition and those that had previously experienced the birth of more than four stillborn piglets. These sows were randomly assigned to three different groups: C ($n = 31$), VC ($n = 29$) and VC+Fe ($n = 30$). The distribution of parities among these three groups closely matched the parity distribution observed on the farm (parity 1–2: 40 %, parity 3–5: 24 %, parity ≥ 6: 36 %). The parity 1–2 group consisted of 13, 14 and 12 sows in the C, VC and VC+Fe groups, respectively. The parity 3–5 group included 7 sows for both the C and VC+ Fe groups, and 8 sows in the VC group. Lastly, the parity ≥ 6 group comprised 7 sows in the VC group and 11 in both the VC and VC+Fe groups.

2.4. General management

Every pregnant sow at both stages of the study received 2.2–2.5 kg/day of compound feed containing 13.35 % crude protein, 3.02 % crude fat, 8 % crude fiber, 4.97 % crude ash, 0.71 % lysine, 0.23 % methionine, 0.65 % Ca, 0.22 % Na and 0.48 % P. Iron was also present in the feed: ferrous sulfate monohydrate ($FeSO_4$• H_2O, 75 mg/kg) and iron chelate of amino acids, hydrate (25 mg/kg).

Starting from the 76th day of gestation, pregnant sows in the VC group received a nutritional supplement of vitamin C (Evencit® Quality, Probena, Zaragoza, Spain: 6.03 % vitamin C, 0.8 % min bioflavonoids, 3.5 % polyphenols) The supplement was provided at a rate of 200 gs per metric ton of compound feed.

In contrast, from the 76th day of gestation, pregnant sows in the VC+Fe group received a daily nutritional supplement comprising of two components. The first component was ferrous sulfate monohydrate (FeSO4•H2O) with a total iron content of over 31 %, Fe2+ content of over 30 %, less than 2 % w/w free H2SO4, and moisture content below 2.5 %. The compound feed was fortified with an additional 45 mg of this supplement. The second component was vitamin C (Evencit® Quality, Probena, Zaragoza, Spain) provided at the same rate as in the VC group (200 gs per metric ton of compound feed).

Sows in the C group did not receive any supplement during the third period of gestation.

2.5. Hemoglobin concentration analysis

The blood Hb concentration in sows was determined using individual 10-μL blood samples obtained from the caudal vein. The HemocueHb 201+ device (HemoCue AB, ÄNGELHOLM, Sweden) was employed for quantification. Two measurements of individual Hb concentrations were conducted at different time points:

(1) Hb concentration I (Hb I): In all groups, this measurement was performed on day 76 of pregnancy (prior to commencing supplementation, in both the VC and VC+Fe groups).
(2) Hb concentration II (Hb II, antepartum hemoglobin): This measurement was carried out 2–4 days before the estimated date of parturition in all groups.

2.6. Performance data collection

We collected data on the total number of piglets born (TBP) and their individual birth weights (in kilograms) from three different groups: C, VC, and VC+Fe. Specifically, we recorded 376 piglets from the C group, 403 piglets from the VC group, and 392 piglets from the VC+Fe group.

2.7. Statistical analysis

We performed data analysis using IBM SPSS software version 26.0. To examine potential variations in Hb concentration among independent groups (C, VC, and VC+Fe) over time (HbI and HbII), a two-way repeated measures analysis of variance (ANOVA) was conducted. Furthermore, we investigated the influence of groups (C, VC, and VC+Fe) and parity (parity 1–2, parity 3–5, parity ≥ 6) on individual birth weight of piglets, while adjusting for TBP (covariate), using a two-way analysis of covariance (ANCOVA). For all analyses, statistical significance was set at a threshold of $P < 0.05$. When detecting significant differences across multiple comparisons, we employed pairwise comparisons with Bonferroni's correction.

3. Results

3.1. Hemoglobin concentration analysis

Table 1 displays the mean values (± SE, standard error) for Hb I and II concentrations in the three groups. We observed a significant two-way interaction between the group and phase regarding blood Hb concentration ($P = 0.002$). In the C group, there was a significant decrease in Hb II concentration ($P = 0.024$). Conversely, in the VC group, we did not observe any significant difference ($P = 0.235$), while the VC+ Fe group exhibited a significant increase in Hb II concentration ($P = 0.031$). Regarding Hb I, we did not find any significant differences among the groups ($P = 0.127$), suggesting initial comparability between these groups. However, there were significant differences in Hb II ($P = 0.005$) between groups, with the VC group demonstrating higher Hb II values compared to the C group, whereas the VC+ Fe group did not differ significantly from the other groups.

3.2. Performance data analysis

Table 2 presents the adjusted means (±SE) for individual birth weight of piglets (kg) while controlling for TBP, considering the effects of group and mother's parity. An important finding was the significant interaction between parity and group on birth weight ($P = 0.001$), indicating that the impact of group on the birth weight of piglets varied depending on the mother's parity.

Specifically, the effect of group on individual birth weight of piglets was found to be statistically significant for parity 1–2 ($P < 0.001$) and parity 3–5 ($P = 0.016$). In the case of parity 1–2, both the VC and VC+Fe groups exhibited significantly higher birth weights compared to the C group. On the other hand, for parity 3–5, the C group did not differ significantly from the other groups, but the VC group demonstrated a significantly higher birth weight compared to the VC+Fe group.

Moreover, it is worth noting that regardless of the group, the mother's parity had a significant influence on birth weight ($P < 0.001$) in all cases

4. Discussion

This field study was conducted under commercial conditions, involving a significant number of animals, which enhances the reliability and validity of the conclusions drawn. This aspect stands out as the greatest strength of the study. However, it is important to note that the study focused solely on two parameters, namely Hb levels in sows and piglet birth weight. This limited scope poses a weakness; unfortunately, data regarding blood Fe concentrations and weaning weight of piglets were not collected.

4.1. Hb concentration analysis

Regarding Hb I, we observed a significant decrease in Hb II within the C group. This decrease could be attributed to either iron deficiency or impaired iron absorption caused by a lack of vitamin C. However, when vitamin C supplementation was administered, the decline in Hb II was prevented in the VC group. Interestingly, in the VC+Fe group where both vitamin C and ferrous sulphate were supplemented, Hb II increased compared to its initial value (Hb I). In both groups, the low antepartum hemoglobin levels (HbII) associated with iron deficiency anemia were effectively addressed, thereby improving the hematological status of the pregnant sow. However, we did not observe a distinct advantage of VC+Fe over VC in terms of Hb II.

It is worth noting that pregnant and lactating sows are particularly susceptible to oxidative damage due to metabolic changes during these productive phases. The concentration of vitamin C in their serum remains constant until approximately the 60th-80th day of pregnancy but gradually declines thereafter and during lactation (Mahan et al., 2007). Our findings further emphasize the importance of incorporating at least vitamin C supplementation as part of the preparation for parturition.

Table 1
Means (± SE) for blood Hb concentration (g/dl) in the C, VC and VC+Fe groups at times I and II (Hb I and Hb II).[A,B and a,b]

Group	Hb I Mean ± SE (g/dl)	Hb II Mean ± SE (g/dl)	*P*-value
C ($n = 31$)	11.09 ± 0.28[A, a]	10.02 ± 0.37 [B, a]	0.024
VC ($n = 29$)	10.94 ± 0.32[A, a]	11.36 ± 0.28 [A, b]	0.235
VC + Fe ($n = 30$)	10.31 ± 0.26[A, a]	10.91 ± 0.19 [B a,b]	0.031
P-value	0.127	0.005	

[A,B] : different capital letters indicate significant differences between Hb I and II within group (row).
[a,b] : different lowercase letters indicate significant differences among groups in one period (column).

Table 2
Adjusted means (±SE) and n for birth weight (kg) of piglets, while adjusting by number of total born piglets (TBP) for group and mother's parity (variables in the model were evaluated in TBP = 14,82piglet/litter).[A,B and a,b]

Group	Parity 1–2 Mean ± SE(kg); n	Parity 3–5 Mean ± SE(kg); n	Parity≥6 Mean ± SE(kg); n	*P*-value
C ($n = 376$)	1.20 ± 0.028 [A, a]; 147	1.51 ± 0.036 [B a,b]; 87	1.53 ± 0.029[B, a]; 142	<0.001
VC ($n = 403$)	1.34 ± 0.026 [A, b]; 180	1.52 ± 0.034[B, b]; 134	1.57 ± 0.036 [B, a]; 89	<0.001
VC+ Fe ($n = 392$)	1.35 ± 0.027[A, b]; 163	1.39 ± 0.035 [A, a]; 93	1.53 ± 0.030 [B, a]; 136	<0.001
P-value	<0.001	0.016	0.600	

[A,B] : different capital letters indicate significant differences among parities within group (row).
[a,b] Different lowercase letters indicate significant differences among groups within parity (column).

4.2. Performance analysis

A positive effect of supplementation on individual birth weight of piglets was demonstrated only for younger sows (parity 1–2), with similar effects for both the VC and VC+Fe groups. Petrichev and Bambova (2005) found that Fe supplementation at the end of pregnancy, when the fetus grows most rapidly, improves piglet weight. Also, Campos et al. (2012) observed fetal growth retardation and lower birth weights in piglets when the sow lacked a nutrient supply during this period.

Improving the supply of iron (Fe) to the sow during different stages of pregnancy has varying effects. In pigs, as with most mammals, the demand for iron significantly increases in the final period of pregnancy (Mazgaj et al., 2020). During the second half of pregnancy, there is rapid embryonic growth, leading to an increased nutrient requirement for the sow (Mc Pherson et al., 2004). Additionally, the absorption of trace elements decreases in the last week of pregnancy (Kirchgessner et al., 1980). Consequently, providing adequate iron supply in the last trimester can enhance piglet weight (Petrichev and Bambova, 2005). Since pig fetuses solely rely on maternal supply for iron, ensuring sufficient availability of iron in sows becomes crucial to meet the growing fetuses' needs (Buhi et al., 1982)

In our study, the benefits of supplementing the feed with vitamin C + iron or vitamin C alone were only evident in young sows (parity 1 and 2). This may be attributed to the increase in Hb II levels we previously observed. Once again, no clear advantage of VC+Fe over VC supplementation was observed. Young sows exhibit an immature physiology and have higher amino acid and mineral requirements compared to multiparous sows; they also have lower body weight, resulting in lower feed consumption and a greater need for lean tissue growth (Koketsu et al., 2017). Therefore, supplementation may be recommended more for females in their first and second reproductive cycles rather than mature females.

5. Conclusions

Pregnant sows have significantly elevated nutritional demands. Our study revealed notable benefits of supplementation in both the vitamin C (VC) and vitamin C plus iron (VC+Fe) groups during the third trimester, specifically with regards to Hb levels. Furthermore, we observed a substantial increase in individual birth weights of piglets, indicating positive effects specifically in younger sows (cycles 1–2). Interestingly, our findings did not demonstrate a discernible advantage in incorporating additional iron beyond the existing levels found in the pregnancy feed. Thus, it appears that vitamin C supplementation alone would suffice to yield favourable outcomes.

Ethical animal research

The experiments complied with the precepts of animal welfare, as approved by the Committee of Ethics in Animal Experimentation of Universidad de Zaragoza (protocol no. PI46/21).

Author statement

We are sending you the revised version of our manuscript entitled "Effect of vitamin C and iron supplementation in pregnant hyper-prolific sows" (Short communication, LIVSCI-D-22–00,153 R1). All the comments from both reviewers have been carefully considered and suitable rebuttals for comment not addressed are enclosed in the attached answers for reviewers. The English language has been carefully revised.

Neither the manuscript nor any parts of its content are currently under consideration or published in another journal. All authors contributed to the preparation of the manuscript and gave approval of the revised manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used [OpenAI/Chat GPT] in order to improve grammar and style. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

CRediT authorship contribution statement

Beatriz Aznar: Investigation, Methodology, Writing – original draft. **Juan Grandia:** Conceptualization, Visualization, Writing – review & editing. **M.Teresa Tejedor:** Data curation, Validation, Software, Writing – original draft. **M.Victoria Falceto:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

At time when this work was carried out, the author Beatriz Aznar was an employee of the company manufacturing the Vitamin C complement that is mentioned in the manuscript (Probena, Zaragoza, Spain), but this company had no significant financial contribution to this work. The rest of authors declare no conflict of interests.

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