Effect of Parenteral Antioxidant Supplementation During the Dry Period on Postpartum Glucose Tolerance in Dairy Cows

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Background: Exacerbated postparturient insulin resistance (IR) has been associated with several pathologic conditions in dairy cattle. Oxidative stress (OS) plays a causative role in IR in humans, and an association, but not direct relationship, between OS and IR recently has been reported in transition dairy cattle.

Hypothesis: Supplementation with antioxidants shortly before calving improves glucose tolerance after parturition in dairy cattle.

Animals: Ten late-pregnant Holstein cows entering their 2nd to 5th lactation.

Methods: Randomized placebo-controlled trial: 15 ± 2 days before expected calving, the treatment group received an injection of DL-alpha-tocopheryl acetate at a dosage of 6 mg/kg body weight (BW) and 0.06 mg/kg BW of sodium selenite, and the control group was injected with isotonic saline. During the first week after calving, both groups underwent glucose tolerance testing (0.25 g glucose/kg BW). Commercial assays were used to quantify the concentrations of glucose, insulin, nonesterified fatty acids (NEFA), beta-hydroxybutyrate, and markers of redox status in blood. Data were analyzed using the Mann–Whitney U-test (α = 0.05).

Results: Supplemented cows showed a lower risk for OS, as reflected by a lower OS index (P = 0.36), different areas under the curve for the concentrations of glucose (P < .01), insulin (P = .043), and NEFA (P = .041), more rapid elimination rates (P = .080, <.01 and .047 respectively), and shorter half-lives (P = .040, <.01 and .032) of these metabolites.

Conclusions and Clinical Importance: Supplementation with antioxidants before calving resulted in greater insulin sensitivity after calving, thereby suggesting the role of OS in the development of IR in cattle and the potential benefits of antioxidant supplementation in minimizing the consequences of negative energy balance.

Key words: Inflammation; Insulin resistance; Oxidative stress; Transition period; Vitamin E.

As dairy cows transition from late pregnancy to the onset of lactation, they are faced with marked and sudden metabolic and endocrine changes that negatively impact their performance and health status. Insulin plays a pivotal role in the partitioning processes that take place to support lactation. Cows undergo a period of insulin resistance (IR) before calving to support fetal glucose needs as well as after calving to prioritize the insulin-independent uptake of glucose by the mammary gland. A prolonged IR state has been related to several pathologic processes, including economically important postpartum conditions such as displaced abomasum or decreased fertility as a consequence of enhanced lipolysis. The mechanisms causing IR are not fully understood, but this period of IR has physiologic similarities to human type I and type II diabetes, with the major difference being that cows have low glucose concentrations. In human type II diabetes, strong evidence supports that oxidative stress (OS), the imbalance between pro-oxidant production and antioxidant capacity, plays a causative role in the development of IR, and antioxidant supplementation can be used to decrease the consequences of IR. It is now well known that dairy cattle experience OS after calving, and antioxidant supplementation can be used to decrease the consequences of IR.
and antioxidant supplementation can diminish the harmful effects of excessive pro-oxidant production.\textsuperscript{13} We recently found a significant association between oxidant status and whole-body insulin sensitivity, measured by means of surrogate indices, in periparturient dairy cattle.\textsuperscript{14} We therefore hypothesized that antioxidant supplementation before calving may impact glucose homeostasis (assessed by means of intravenous glucose tolerance testing [IVGTT]) after calving. Hence, our study aimed to establish a causal relationship between oxidant status and insulin sensitivity in dairy cattle during the transition period.

Material and Methods

A randomized placebo-controlled study was used. The protocols of this study were approved by the Bioethical Committee of the University of Santiago de Compostela (Spain), and the animals were enrolled with owner consent.

Animals, Nutrition and Husbandry

Ten nonlactating, late-pregnant Holstein cows from the same commercial herd, located in Meira (northwest Spain), were used in this study. Selection criteria included: parity (entering their 2nd or greater lactation), milk production in the preceding lactation (9000 to 9500 kg), body condition score (3 to 3.5, on a 1 [lean] to 5 [obese] scale as previously described\textsuperscript{15}), and proximity in their expected calving date. Cows in both groups were maintained under identical conditions throughout the study. Animals were kept in a free-stall barn with concrete stalls and fed a total mixed ration corresponding to the National Research Council (NRC)\textsuperscript{16} to meet or exceed the NRC\textsuperscript{16} scale as previously described\textsuperscript{15}, and proximity in their expected calving date. Cows in both groups were maintained under identical conditions throughout the study. Animals were kept in a free-stall barn with concrete stalls and fed a total mixed ration (Table 1), delivered once daily at 9:00 AM and formulated according to the National Research Council (NRC)\textsuperscript{16} to meet or exceed their requirements. Lactating animals were milked twice daily and cows were dried-off 60 days before their expected calving date.

Table 1. Ingredients and chemical composition of the diets fed to the cows in the different stages of the study.

| Diet Composition (kg dry matter/cow/d) | Lactating Diet | Pre-Fresh Diet |
|--------------------------------------|----------------|----------------|
| Total dry matter offered             | 21.7           | 13.2           |
| Grass silage                         | 8.6            | 5.8            |
| Concentrate\textsuperscript{a}       | 13.10          | 7.4            |
| Nutrient analysis                    |                |                |
| Dry matter (%)                       | 43.7           | 36.7           |
| Crude protein (% DM)                 | 16.7           | 9.9            |
| Neutral detergent fiber (% DM)       | 35.4           | 61.6           |
| Acid detergent fiber (% DM)          | 21.9           | 39.3           |
| Starch (% DM)                        | 21.6           | 13.8           |
| Ether extract content (% DM)         | 6.2            | 2.7            |
| Ashes (% DM)                         | 8.4            | 7.1            |
| EN\textsubscript{j} (MJ/kg DM)       | 6.76           | 5.19           |

\textsuperscript{a}Concentrate composition (% as fed): corn (49.7), soybean meal (19.9), rapeseed meal (11.5), barley (7.5), vegetable soapstock (3.6), beet molasses (2.50), calcium carbonate (1.6), calcium bicarbonate (1.5), sodium chloride (.9), and vitamin/mineral premix (.4). The vitamin/mineral premix contained: 16650 IU/kg vitamin A, 4350 IU/kg vitamin D3, 66.65 mg/kg vitamin E (\textsuperscript{a}-tocopherol), 120 mg/kg Zn (oxide), 50 mg/kg Mn (oxide II), 27.5 mg/kg Cu (sulfate), 7.6 mg/kg Fe (sulfate), 2.0 iodine (potassium iodide), 1.3 mg/kg Co (carbonate), and .5 mg/kg Se (sodium selenite).

Treatment Allocation

Animals were randomly allocated to treatment or control groups using the random function of Excel.\textsuperscript{9} A blood sample was collected 15 ± 2 days before expected calving by coccygeal venipuncture into evacuated tubes without anticoagulant,\textsuperscript{10} and animals in the supplementation group subsequently received an IM injection of a commercial product\textsuperscript{a} at a dosage of 6 mg/kg body weight (BW) of DL-alpha-tocopheryl acetate (equivalent to 6 IU/kg BW of vitamin E) and 0.06 mg/kg BW of sodium selenite, whereas cows in the control group were injected with isotonic sterile saline solution.\textsuperscript{11} BWs for dose calculation were adjusted estimating the weight of the conceptus according to NRC\textsuperscript{16} using an estimated calf birth weight of 40 kg. The farm personnel, but not the investigators, were blinded to group allocation. Because of longer gestation lengths than expected, the interval between treatment and calving ranged from 9 to 19 days (mean ± SD: 16 ± 4.76).

Intravenous Glucose Tolerance Test

Between days 3 to 7 after calving, animals in both groups were subjected to IVGTT around 3:00 pm, thereby allowing 6 hour between when the ration was offered and the infusion of glucose to decrease any potential interference in blood metabolite clearance patterns. Cows were restrained in the feedbunk headlocks and the feed was removed from their access. A 14-gauge x 8 cm catheter with a 250 mL/min capacity\textsuperscript{12} was inserted in either the right or the left jugular vein. Cows were allowed to rest for 15 minute after insertion of the catheter until blood sampling started. Stress was avoided as much as possible and cows generally appeared relaxed and continued to ruminate during the test. Blood samples were collected at −10, −5, 5, 10, 20, 30, 45, 60 and 90 minutes after the infusion of 0.25 g/kg BW of glucose.\textsuperscript{12} The infusion of glucose was completed in 3 to 4 minutes. After infusion, the catheters were irrigated with 10 mL of sterile saline\textsuperscript{13} and the first 5 mL of blood discarded from the first collection. Samples were collected into tubes without anticoagulant and tubes containing fluoride heparin.\textsuperscript{14}

Laboratory Analysis

Samples were transported under refrigeration to the laboratory, where they were centrifuged at 2000 x g for 20 minutes within 2 hour after collection and the supernatant serum or plasma was harvested, aliquoted into 1.7 mL microcentrifuge tubes\textsuperscript{15} and stored at −80°C pending analysis within 3 months of collection. Commercially available kits were used for analysis. Plasma was analyzed for glucose concentration,\textsuperscript{16} whereas serum was used to measure the concentration of nonesterified fatty acids\textsuperscript{17} (NEFA) and beta-hydroxybutyrate\textsuperscript{18} (BBHA). Biomarkers of oxidant status were measured at enrollment into the study and in the basal IVGTT samples. Reactive oxygen species\textsuperscript{19} (ROS) were quantified in serum samples as markers of pro-oxidants. The assay employed determines hydroperoxides (breakdown products of lipids and other organic substrates generated by oxidative attack of ROS) through their reaction with the chromogen N,N-diethyldihydroxyphenylenediamine. This assay previously has been validated against electron spin resonance.\textsuperscript{17} Results are expressed in arbitrary ‘Carratelli units’ (Carr.U), with 1 Carr.U corresponding to the oxidizing power of 0.08 mg H\textsubscript{2}O\textsubscript{2}/dL. Total serum antioxidant capacity (SAC) also was quantified using a commercial assay.\textsuperscript{20} This test exploits the capacity of a concentrated solution of hypochlorous acid (HClO) to oxidize the complete pool of antioxidants in serum (albumin, bilirubin, uric acid, thiol groups, vitamins, glutathione, glutathione peroxidase, super-
oxide dismutase, catalase, and other compounds). Thus, SAC considers the cumulative action of all the antioxidants present in serum, rather than simply the sum of measurable antioxidants. Results are expressed as μmol HClO/mL. The oxidative stress index (OSi) was calculated as ROS/SAC.\(^1\) Thus, an increase in the ratio indicates a higher risk for OS because of an increase in ROS production, defensive antioxidant consumption, or both.

These analytical determinations were performed in duplicate on a biochemistry autoanalyzer\(^3\) calibrated against a multipoint calibrator.\(^4\) Physiologic and pathologic control sera, as well as an in-house reference sample, were analyzed alongside the samples for quality control. Duplicated serum samples also were analyzed for insulin using a bovine-specific ELISA kit,\(^5\) which has a limit of detection of 0.025 μg/L. Two samples fell below this limit and were assigned a concentration of 0.025 μg/L. The intra-assay coefficients of variation for all the determinations were below 5%, with all samples analyzed in the same run.

**IVGTT Data Processing**

Basal concentrations for the studied analytes were determined as the mean concentration of the 2 blood samples taken before glucose infusion (~10 and ~5 minutes samples). The area under the curve (AUC) of glucose, insulin, NEFA, and BHBA were computed with the trapezoidal method as the total increment of these metabolites above (below for NEFA and BHBA) basal concentrations during the 90 minutes after infusion. Peak and nadir concentrations of these analytes also were determined. Elimination rates and times to reach half-maximal \((T_{1/2})\) and basal \((T_{\text{basal}})\) concentrations for glucose, insulin, NEFA, and BHBA were computed with the following formulas, as previously described\(^3\):

\[
\text{Elimination rate} = \frac{[\ln(t_2) - \ln(t_1)]}{(t_0 - t_1) \times 100}
\]

\[
T_{1/2} = \frac{\ln(2)}{\text{Elimination rate}} \times 100
\]

\[
T_{\text{basal}} = \frac{[\ln(t_0) - \ln(t_1)]}{(t_0 - t_1) \times 100}
\]

In these formulas, \([t_0]\) is the concentration of the metabolite at time \(a\) \((t_a)\) and \([t_1]\) is the concentration of metabolite at time \(b\) \((t_b)\).

**Statistical Analyses**

No assumptions for normality of data were made because of small sample size. All variable concentrations were analyzed with the Mann–Whitney \(U\)-test using SPSS software\(^6\) and expressed as medians. Statistical significance was declared at \(P < 0.05\), and values of \(P\) between .05 and .10 were considered a trend toward significance.

**Results**

No statistically significant differences between the control and supplemented groups were observed for the distribution of parity (mean ± SD: 2.4 ± 0.54 and 2.8 ± 1.07, respectively, \(P = .48\)), the number of days open before conception (123.2 ± 32.78 and 127.8 ± 85.37, \(P = .88\)), or adjusted BW at treatment allocation (637.4 ± 29.40 and 639.20 ± 37.55 kg, respectively, \(P = .91\)). Milk yields in the day preceding the IVGTT were similar between both groups (28.7 ± 4.47 vs. 29.6 ± 7.45 L, \(P = .37\)), as were the means ± SD of days postpartum at the time of IVGTT (4.8 ± 0.98 vs. 5.0 ± 1.09, \(P = .69\)). No differences between groups in the concentration of the studied analytes were found at the time of enrollment (Table 2), whereas during the IVGTT basal measurements, only the SAC and OSi differed, being higher and lower, respectively, in the supplemented group (Table 2), thereby indicating a decreased risk for OS.

Responses to the IVGTTs are quantified in Table 3. Cows supplemented with vitamin E and selenium showed a smaller glucose AUC, lower nadir concentration, and shorter half-life. There was no difference between groups in maximum concentration during IVGTT (Fig 1A, \(P = .64\)) or \(T_{\text{basal}}\) (\(P = .77\)) for glucose during IVGTT. Similar to the changes observed in glucose, the insulin AUC, insulin minimum concentration, and insulin half-life were decreased in supplemented cows. Insulin secretion in response to glucose infusion (peak concentration) was not affected by treatment (Fig 1B, \(P = .29\)), but supplemented cows had more rapid insulin clearance (elimination rate) and a shorter

| Table 2. | Concentration of the studied serum/plasma analytes at the different time points of the study. |

| Variable    | Units     | Control Group (n = 5) | Supplemented Group (n = 5) | P-Value | Control Group (n = 5) | Supplemented Group (n = 5) | P-Value |
|-------------|-----------|-----------------------|---------------------------|---------|-----------------------|---------------------------|---------|
|             |           | Median                | IQR                       |         | Median                | IQR                       |         |
| ROS         | Carr.U.   | 97.4                  | 51.1                      | 104.2   | 52.5                  | NS                        |         |
| SAC         | μmol HClO/mL | 222.4                | 217.6                      | 327.1   | 208.8                  | NS                        |         |
| OSI         | —         | 0.47                  | 0.29                      | 0.38    | 0.34                  | NS                        |         |
| Glucose     | mg/dL     | 76.2                  | 23.4                      | 76.7    | 14.7                  | NS                        |         |
| Insulin     | μg/L      | 0.46                  | 0.39                      | 0.29    | 0.17                  | NS                        |         |
| NEFA        | mEq/L     | 0.15                  | 0.22                      | 0.23    | 0.04                  | NS                        |         |
| BHBA        | mg/dL     | 8.35                  | 7.73                      | 8.25    | 8.04                  | NS                        |         |

ROS, Reactive oxygen species; SAC, Serum antioxidant capacity; OSi, Oxidative stress index; NEFA, nonesterified fatty acids; BHBA, beta-hydroxybutyrate; IVGTT, intravenous glucose tolerance test; DIM, Days in milk; IQR, interquartile range.

Differences between groups were assessed using the Mann–Whitney \(U\)-test. NS, Nonsignificant \((P > .10)\).
Tbasal, requiring only 44% of the time required by non-supplemented animals to reach basal insulin concentration after glucose infusion. Differences in fatty acid metabolism also were observed between groups (Fig 1C). Supplemented cows had larger NEFA AUC, a faster NEFA elimination rate, and a decreased NEFA half-life. However, neither the peak nor nadir concentrations of NEFA were different between groups. In addition, the metabolism of ketones was similar between the 2 groups (Fig 1D), where only the nadir concentration of BHBA tended to be lower in supplemented animals (P = .086).

### Discussion

In humans suffering from diabetes, OS plays a causal role in the development of IR, 7,19 decreasing insulin biosynthesis and release. However, this direct relationship has hitherto not been proven in periparturient cattle, although from epidemiologic data we recently reported a significant association between markers of OS and IR in these animals. 14 OS is well known in cattle as an underlying cause of dysfunctional inflammatory and host immune responses around the time of calving, thereby increasing cows’ susceptibility to health disorders. 20 Indeed, antioxidant supplementation has shown an overall beneficial effect on the health status and performance of cows. 13 OS links nutrient metabolism with inflammatory responses in transition cattle 12 and therefore, supplementation with vitamin E and selenium precalving has the potential to alter the metabolic response of the animals to an IV infusion of glucose. Vitamin E (α-tocopherol) is a potent lipid-soluble, chain-breaking antioxidant, 21 and selenium also exerts antioxidant functions both directly and as a cofactor for selenoproteins. 22 Hence, the parenteral administration of these 2 compounds increased the SAC of the animals (Table 2), thereby decreasing the risk for OS in the supplemented animals when they underwent IVGTT, as shown by the lower OSi values. Also, more individual variability was observed in SAC before treatment application than at IVGTT. Cows managed under identical conditions show high individual variability in their physiologic adaptation to metabolic stress around calving. 23 Yet, cows at the onset of lactation typically show decreased antioxidant capacity, 13 which could explain the decreased variability in control cows in the first week of lactation. On the other hand, supplemented cows all received the same dose of vitamin E and selenium at a similar time point, which contributes to a similar total antioxidant potential.

### Table 3. Comparison of the response to the IVGTT between supplemented and nonsupplemented animals.

|                      | Control Group (n = 5) | Supplementation Group (n = 5) | P-Value |
|----------------------|-----------------------|-------------------------------|---------|
|                      | Median | IQR             | Median | IQR             |         |
| **Area under the curve** |         |                  |         |                  |         |
| Glucose (mg/dL × 90 min) | 6615.6 | 1401.6          | 4047.6 | 1897.2          | <.01    |
| Insulin (µg/L × 90 min)  | 108.4  | 31.8            | 74.9   | 44.8            | .043    |
| NEFA (mEq/L × 90 min)   | −16.9  | 17.0            | −42.2  | 28.7            | .041    |
| BHBA (mg/dL × 90 min)   | −181.4 | 323.7           | −203.1 | 348.4           | NS      |
| **Peak concentration**  |         |                  |         |                  |         |
| Glucose (mg/dL)        | 243.6  | 106.8           | 278.4  | 200.4           | NS      |
| Insulin (µg/L)         | 3.30   | 2.06            | 3.49   | 1.83            | NS      |
| NEFA (mEq/L)           | 0.47   | 0.63            | 0.50   | 0.35            | NS      |
| BHBA (mg/dL)           | 17.32  | 10.31           | 11.34  | 8.35            | NS      |
| **Nadir concentration**|         |                  |         |                  |         |
| Glucose (mg/dL)        | 81.6   | 52.8            | 45.6   | 8.4             | .029    |
| Insulin (µg/L)         | 0.74   | 0.32            | 0.22   | 0.10            | <.01    |
| NEFA (mEq/L)           | 0.21   | 0.38            | 0.17   | 0.19            | NS      |
| BHBA (mg/dL)           | 12.37  | 7.63            | 7.53   | 5.57            | .086    |
| **Elimination rate (%/min)** |        |                  |         |                  |         |
| Glucose                | 1.83   | 1.05            | 2.50   | 0.91            | .080    |
| Insulin                | 1.48   | 1.79            | 3.36   | 1.43            | <.01    |
| NEFA                   | 1.38   | 1.25            | 2.82   | 1.84            | .047    |
| BHBA                   | 0.56   | 0.42            | 0.84   | 0.53            | NS      |
| **Time to reach half-maximal concentration (min)** |        |                  |         |                  |         |
| Glucose                | 38.6   | 28.7            | 25.8   | 17.1            | .040    |
| Insulin                | 46.7   | 92.0            | 20.6   | 6.03            | <.01    |
| NEFA                   | 40.6   | 21.3            | 24.2   | 10.8            | .032    |
| BHBA                   | 124.0  | 92.2            | 98.0   | 63.7            | NS      |
| **Time to reach basal concentration (min)** |        |                  |         |                  |         |
| Glucose                | 70.0   | 30.0            | 85.0   | 37.5            | NS      |
| Insulin                | 85.0   | 7.5             | 70.0   | 20.0            | <.01    |

BHBA, beta-hydroxybutyrate; IQR, interquartile range; NEFA, nonesterified fatty acids. Comparisons between groups were made using the Mann–Whitney U-test. NS, Not significant (P > .10).
The dose of glucose administered during the IVGTT differed from some previous studies, which employed larger and smaller doses than used in this study. We selected a dosage of 0.25 g glucose per kg BW to facilitate the comparison of results, because this was the same or a similar dosage to that used in the majority of previous studies. 

Higher glucose tolerance was found in the supplemented animals, with lower glucose AUC and T1/2. Increased glucose elimination rates, decreased half-life and decreased AUC are thought to involve increased insulin sensitivity. This assumption is further supported by the smaller insulin AUC, a quicker elimination rate, and shorter T1/2 and T basal for insulin found in supplemented animals. Similarly, a higher insulin AUC in control animals clearing the same dose of glucose indicates a higher degree of IR.

In addition, differences in fatty acid metabolism were observed in the response to IVGTT in this study. In accordance with previous studies, NEFA concentrations reached their nadir at approximately 45 minutes, representing rapid inhibition of lipolysis by insulin. Supplemented cows had a more rapid NEFA elimination rate after glucose infusion (Table 3), higher NEFA AUC, and a shorter NEFA half-life, thereby suggesting that supplemented cows had lower IR related to lipid metabolism than did nonsupplemented cows. Conversely, regarding the response of NEFA to the IVGTT, no differences in the metabolism of BHBA after glucose infusion were observed between the 2 groups. However, concentrations of NEFA and BHBA do not correlate well, because the synthesis of ketone bodies does not depend only on energy balance. Therefore, the greater decrease in serum NEFA may not directly translate to a greater decrease in the concentration of BHBA.

To the best of the authors’ knowledge, ours is the first study to investigate the effect of supplementation with vitamin E and selenium, the most widely used antioxidants included in the diets of dairy cows, on glucose tolerance during early lactation. However, 2 previous reports investigated the effect of chromium supplementation, which has some antioxidant effects in cattle, on the response to IVGTT in cows. These studies found differences in glucose elimination rates,
but not in the clearance of NEFA. However, despite the limited antioxidant potential of chromium, its role in metabolism is believed to be through the glucose tolerance factor,\textsuperscript{35} enhancing glucose uptake by cells. Therefore, it is not surprising that these studies reported improved glucose clearance, but no changes in the NEFA response to the IVGTT.

Inflammation around the time of calving has gained much attention in recent years.\textsuperscript{36} The nuclear factor kappa B (NF-\textit{kappa} B) pathway is a pro-inflammatory signaling pathway responsible for provoking IR.\textsuperscript{37} This pathway can be activated by OS in cattle during times of negative energy balance.\textsuperscript{38,39} In addition, endoplasmic reticulum stress, present in the liver of high-yielding dairy cows,\textsuperscript{40} also activates inflammation via the NF-\textit{kappa} B pathway.\textsuperscript{41} Hence, the lower IR observed in supplemented cows may be a consequence of the down regulation of these pathways because of increased antioxidant capacity. However, as a consequence of the tight interplay among nutrient metabolism, OS, and inflammation in dairy cattle,\textsuperscript{12} several other factors may play key roles in the development of IR. In dairy cows, which must also be taken into consideration when designing nutritional interventions to control IR and the associated enhanced lipolysis.

The use of the IVGTT to assess insulin sensitivity implies normal insulin secretion after glucose administration and assumes similar insulin secretion among animals, which may not always be the case.\textsuperscript{1} The IVGTT, however, is considered a good method for assessing IR in cattle given its practicality and agreement with the hyperinsulinemic euglycemic clamp, the gold standard test.\textsuperscript{26} The major limitation of this study was the small sample size, as there were only 5 animals per study group. Nevertheless, this number was sufficient for showing statistical differences in the response to IVGTT, although the basal metabolic status of the animals was not affected by the supplementation. Animals in this study were not supplemented with any dietary antioxidants aside from the limited amount contained in preserved forages,\textsuperscript{16} and therefore the improved responses observed in this study might also be in part because of some antioxidant deficiency during the dry period. Hence, further studies should investigate whether antioxidant therapy ameliorates the degree of IR beyond the first week postcalving, as well as the impact that antioxidant supplementation can have on the metabolic and health status of cows.

**Conclusions**

Cows supplemented parentally with antioxidants (vitamin E and selenium) before calving showed improved insulin sensitivity during the first week of lactation, thereby supporting an effect of OS on the development of IR in dairy cows. Further studies should investigate the effects of different supplementation strategies as adjunct therapies to ameliorate the consequences of prolonged IR and its impact on metabolic stress in cows.

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**Footnotes**

\textsuperscript{a} Microsoft Excel 2010, Redmond, MA.  
\textsuperscript{b} BD Vacutainer; Becton, Dickinson and Company, Plymouth, UK.  
\textsuperscript{c} Selevit adultos, Laboratorios SYVA, León, Spain.  
\textsuperscript{d} FisioVet solución para perfusión, B. Braun VetCare SA, Barcelona, Spain.  
\textsuperscript{e} Intraflon 2 catheter IV, Laboratories Pharmaceutiques VYGON, Ecouen, France.  
\textsuperscript{f} GlucosaVet 40 g/100 mL, B. Braun VetCare SA, Barcelona, Spain.  
\textsuperscript{g} 2 mL Glucose Fluoride, Sarsted AG & Co, Nürnberg, Germany.  
\textsuperscript{h} Sarsted AG & Co, Nürnberg, Germany.  
\textsuperscript{i} Glucose-Hexokinase Geron, RAL Tecnica para el Laboratorio, Barcelona, Spain.  
\textsuperscript{j} NEFA H(2) R1+R2 Set, Wako Chemicals GmbH, Neuss, Germany.  
\textsuperscript{k} BHB, Biochemical enterprise, Milan, Italy.  
\textsuperscript{l} d-ROM test, Diacon International, Grosseto, Italy.  
\textsuperscript{m} OXY-Adsorbent test, Diacon International, Grosseto, Italy.  
\textsuperscript{n} CST-240, DIRUI Industrial Co., Ltd, Changchun, China.  
\textsuperscript{o} Biocal; RAL Tecnica para el laboratorio S.A., Barcelona, Spain.  
\textsuperscript{p} Gernorm; RAL Tecnica para el laboratorio S.A., Barcelona, Spain.  
\textsuperscript{q} Gerpath; RAL Tecnica para el laboratorio S.A., Barcelona, Spain.  
\textsuperscript{r} Insulin Bovine ELISA; Merckodia AB, Uppsala, Sweden.  
\textsuperscript{s} SPSS v.20 for Windows, IBM, Chicago, IL.

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**Conflict of Interest Declaration:** Authors declare no conflict of interest.

**Off-label Antimicrobial Declaration:** Authors declare no off-label use of antimicrobials.

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