

# The crucial role of short-chain fatty acids and how phytomolecules influence them



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For optimum health, the content of short-chain fatty acids (SCFAs) is decisive. On the one hand, they act locally in the gut, on the other hand, they are absorbed via the intestinal mucosa into the organism and can affect the whole body. Newer studies in humans show a connection between the deficiency of SCFAs and the occurrence of chronic diseases such as diabetes type 2 or chronic inflammatory gut diseases.

## SCFAs - what are they, and where do they come from?

SCFAs consist of a chain of one to six carbon atoms. They are crucial metabolites primarily generated through the bacterial fermentation of dietary fiber (DF) in the hindgut. However, SCFAs and branched SCFAs can also arise during protein fermentation. Short-chain fatty acids predominantly include acetate, propionate, and butyrate, which together account for over 95% of the total SCFAs, typically in a 60:20:20 ratio.

**Acetate** is produced in two different ways, via the acetyl-CoA and the Wood-Ljungdahl pathways where *Bacteroides* spp., *Bifidobacterium* spp., *Ruminococcus* spp., *Blautia hydrogenotrophica*, *Clostridium* spp. are involved. Additionally, acetogenic bacteria can synthesize acetate from carbon dioxide and formate through the Wood-Ljungdahl pathway (Ragsdale and Pierce, 2021). Acetate counts for more than 50% of the total SCFAs in the colon and is the most abundant one.

**Propionate** can also be produced in two ways. If it is produced via the succinate pathway involving the

decarboxylation of methyl malonyl-CoA, the essential bacteria are Firmicutes and Bacteroides. In the acrylate pathway, lactate is converted to propionate. Here, only some bacteria, such as Veillonellaceae or Lachnospiraceae, participate.

**Butyrate** is produced from acetyl-CoA via the classical pathway by several Firmicutes. However, also other gut microbiota such as Actinobacteria, Proteobacteria, and Thermotogae, which contain essential enzymes (e.g., butyryl coenzyme A dehydrogenase, butyryl-CoA transferase, and butyrate kinase) can be involved. Butyrate can also be produced via the lysine pathway from proteins.

Besides the production of SCFAs from dietary fiber, there is another possibility for the synthesis of SCFAs as well as branched SCFAs – the fermentation of protein in the hindgut. This is something we want to avoid, since it's clear signal of incorrect animal nutrition. It tells us that there is either oversupply of protein or decrease in protein digestion and absorption.

## Which roles do SCFAs play?

SCFAs play a crucial role in the maintenance of gut health. Some benefits originate from these substances' general character, while others are specific to one acid. If we talk about the benefits of all SCFAs, we can mention the following:

1. Primarily, SCFAs are absorbed by the intestine and serve enterocytes as an essential substrate for energy production.
2. By lowering the pH in the intestine, SCFAs inhibit the invasion and colonization of pathogens.
3. SCFAs can cross bacterial membranes in their undissociated form. Inside the bacterial cell, they dissociate, resulting in a higher anion concentration and bactericidal effect (Van der Wielen et al., 2000)
4. SCFAs repair the intestinal mucosa
5. They mitigate intestinal inflammation by G protein-coupled receptors (GPRs).
6. They enhance immune response by producing cytokines such as IL-2, IL-6, IL-10, and TNF- $\alpha$  in the immune cells. Furthermore, they enhance the differentiation of T-cells into T regulatory cells (Tregs) and bind to receptors (Toll-like receptor, G protein-coupled receptors) on immune cells (Liu et al., 2021).
7. SCFAs are involved in the modulation of some processes in the gastrointestinal tract, such as electrolyte and water absorption (Vinolo et al., 2011)

After seeing the general characteristics of short-chain fatty acids, let us take a closer look at the specialties of the single SCFAs.

**Acetate** might play a crucial role in the competitive process between enteropathogens and bifidobacteria and help to build a balanced gut microbial environment (Liu et al., 2021). Additionally, acetate promotes lipogenesis in adipocytes (Liu et al., 2022).

Concerning general health, acetate inhibits, e.g., lung inflammatory response and the reduced air-blood permeability induced by avian pathogenic E. coli-caused chicken colibacillosis (Peng et al., 2021).

**Propionate** is thought to be involved in controlling intestinal inflammation by regulating the immune cells assisting and, consequently, in maintaining the gut barrier. Furthermore, propionate regulates appetite, controls blood glucose, and inhibits fat deposition in broiler chickens (Li et al., 2021).

In a trial conducted by Elsherif et al. (2022), birds fed a diet with 1.5 g sodium propionate/kg showed considerably ( $P < 0.05$ ) longer and wider guts, higher counts of lactobacillus ( $P < 0.05$ ) and no colonization of Clostridium perfringens. The immunological state improved significantly ( $P < 0.05$ ), which could be seen by the higher antibody titers when the birds were vaccinated against Newcastle disease or avian influenza.

**Butyrate** additionally improves the function of the intestinal barrier by regulating the assembly of tight junctions (Peng et al., 2009) and stimulating cell renewal and differentiation of the enterocytes. Butyrate-producing microbes on their side prevent the dysbiotic expansion of potentially pathogenic E. coli and Salmonella (Byndloss et al., 2017; Cevallos et al., 2021) by stimulating PPAR- $\gamma$  signaling. This leads to the suppression of iNOS synthesis and a significant reduction of iNOS and nitrate in the colonic lumen. Furthermore, the microbiota-induced PPAR- $\gamma$ -signaling inhibits dysbiotic Enterobacteriaceae expansion by limiting the bioavailability of oxygen and, therefore, respiratory electron acceptors to Enterobacteriaceae in the colon.

In a trial conducted by Xiao et al. (2023), sodium butyrate enhanced broiler breeders’ reproductive performance and egg quality due to the regulation of the maternal intestinal barrier and gut microbiota. Additionally, it improved the antioxidant capacity and immune function of the breeder hens and their offspring.

## SCFAs’ production can be managed

The extent of production depends on the diet and the composition of the intestinal flora. Nutritional strategies can be taken to regulate the production of short-chain fatty acids by providing dietary fiber and prebiotics, the respective bacteria but also additives in the diet or, on the other, negative way, use of antibiotics.

One example of SCFA-promoting additives is phytomolecules. Ventar D, a blend of diverse gut health-promoting phytomolecules, shows its SCFAs-increasing effect in a trial with Ross 308 broilers.

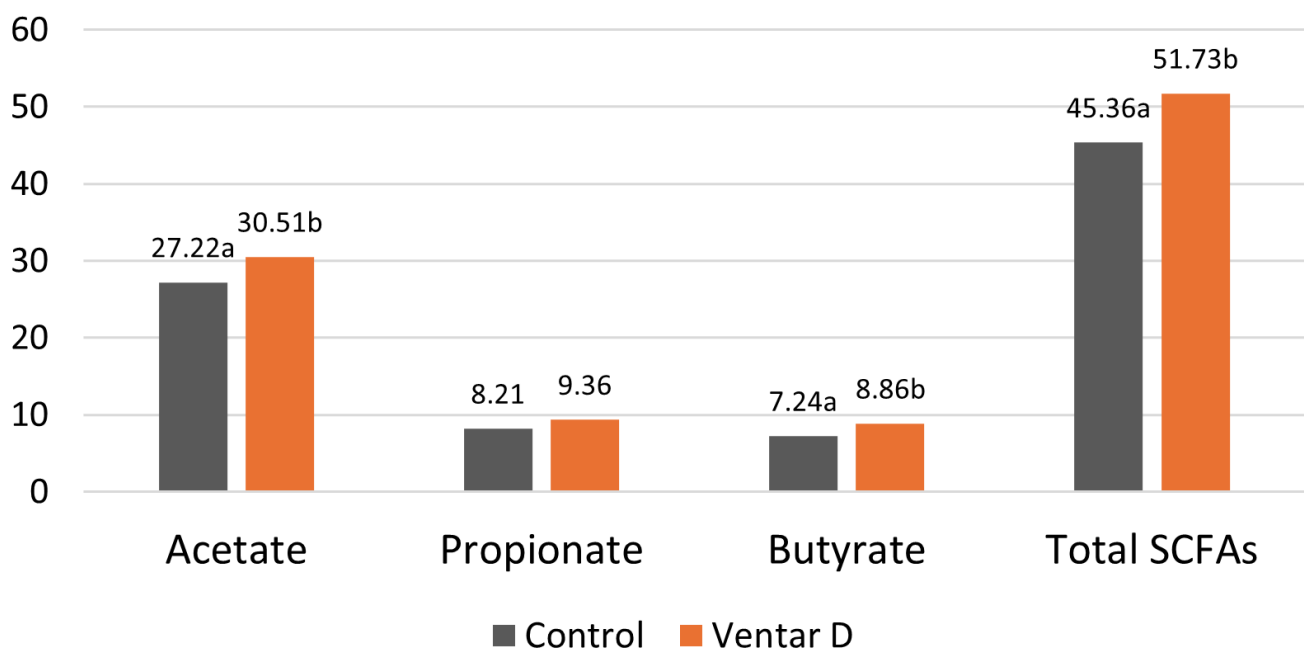
**Trial design:** The 41-day research study was conducted at an R&D farm in Turkey, with 3200 Ross 308 broilers in total. The day-old broiler chicks were randomly divided into two groups with 8 replicates in 16-floor pens (6.5×2 m each), each of 200 chicks (100 males and 100 females). One group was managed as a control group with regular feed formulation, and the other group was supplemented with Ventar D. All the birds were provided feeds and water ad libitum. Temperature, lighting, and ventilation were managed as per Ross 308 recommendation.

| Groups   | Application dose   |                                    |
|----------|--------------------|------------------------------------|
|          | Starter (crumbles) | Grower & Finisher - 1 & 2 (pellet) |
| Control  | No additive        |                                    |
| Ventar D | 100 gm/MT          | 100 gm/MT                          |

All the birds and feed were weighed on days 0, 11, 23, and 41. Dead birds were also weighed, and the feed consumption was corrected accordingly. At the end of the experiment, one male and one female chicken close to the average weight of each pen were separated, weighed, and slaughtered. Short-chain fatty acid (SCFA) concentration in the caecum was measured by gas chromatography (Zhang et al. 2003). Statistical analysis of the data obtained in this study was carried out in the Minitab 18 program using the T-test following the randomized block trial design ( $P \leq 0.05$ ). The research results were subjected to statistical analysis on a pen basis. Mortality results were evaluated with the Chi-square test.

**Results:** Ventar D significantly increased the levels of acetate, butyrate, and total SCFAs. The level of propionate was numerically higher. Additionally, higher final body weights (on average 160 g), improved feed efficiency (6 points), a higher EPEF (33 points), and lower mortality (0.5%) could be asserted in this experiment.

## Influence of Ventar D on the levels of SCFAs



One explanation could be the microbiota-balancing effect of Ventar D. Meimandipour et al. (2010), for example, saw in their study that increased colonization of *Lactobacillus salivarius* and *Lactobacillus agilis* in cecum significantly increased propionate and butyrate formation in caeca.

## Phytomolecules: Balancing intestinal microbiome and increasing healthy SCFAs

By promoting beneficial intestinal bacteria and fighting the harmful ones, phytomolecules drive the microbiome in the right direction and promote the production of short-chain fatty acids. Their gut health-protecting effect, in turn, provides for adequate digestion and absorption of nutrients, leading to optimal feed conversion and growth rates. The support of the immune system and the promotion of the antioxidant capacity additionally enhance the health of the animals. Healthy animals grow better, which ultimately leads to a higher profit for the farm.

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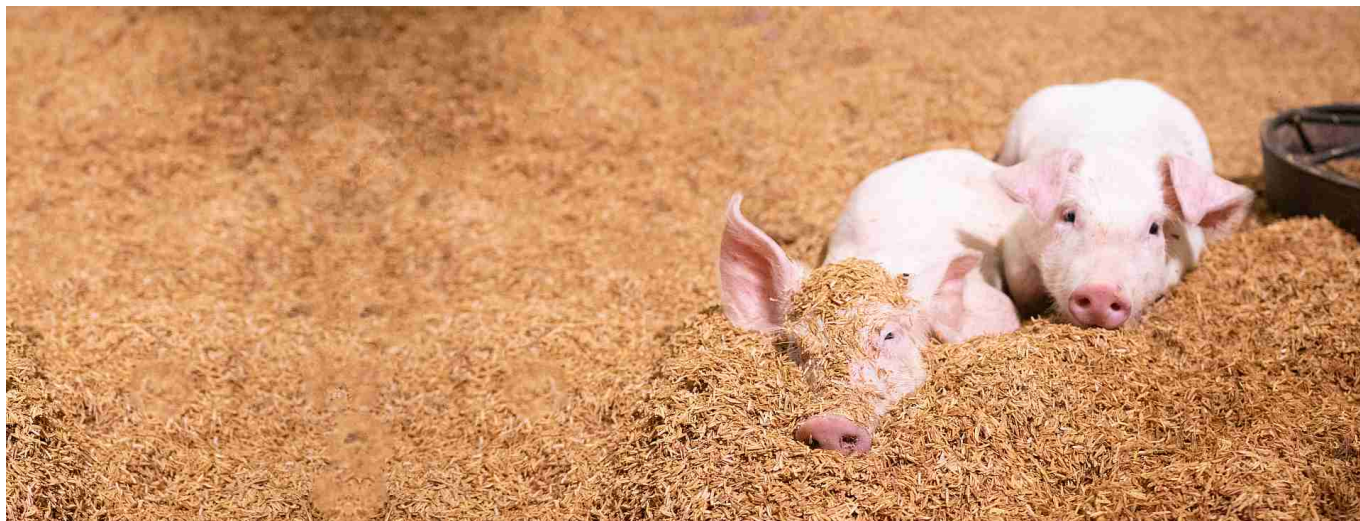
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## Antioxidant benefits in pig feed



*By Technical Team, EW Nutrition*

**In modern swine production, one of the key aspects for success is a balanced diet. This essentially means ensuring that the animal meets its daily nutritional requirements for maintenance, growth, and reproduction. In order to provide an appropriate diet and safe feed for the animals, the sensory and nutritional characteristics of the feed must be preserved and issues like the oxidation of the feed must be avoided.**



This article aims to highlight why oxidation in feed can become a big concern for swine producers, what the problems resulting from oxidation in pig feed are, and present practical solutions to improve feed quality and pig performance by controlling the oxidation.

## **Feed oxidation: What are the dangers?**

In pig diets, various sources of lipids are added to increase caloric density, provide essential fatty acids, improve feed palatability, improve pellet quality, and reduce dust (Keer et al., 2015). Some of the feed ingredients are more susceptible to oxidation because of their physical and chemical characteristics, such as milled grains and ingredients of animal origin and vegetable oils with a high content of polyunsaturated fatty acids.

Oxidative rancidity is a type of lipid deterioration. In the oxidation process, the free radicals react with lipids and proteins and induce cellular and tissue damage.

Some consequences of oxidative deterioration are the destruction of fat-soluble vitamins, supplemental fats, and oils. Preserving these ingredients is crucial because fats and oils provide a high quantity of energy and essential fatty acids. At the same time, vitamins, such as those present in vitamin premixes, are indispensable for optimal animal growth and performance.

The oxidation process also results in by-products with strong unpleasant taste and odor, and even toxic metabolites. In addition, oxidized feed has less protein, amino acids, and energy content. All these factors are relevant when resources, in the current scenario of high prices of feed ingredients and inputs, might be wasted due to poor feed management.

## Performance losses caused by oxidation

Lipid oxidation can incur several losses regarding the pigs' performance. Feeding oxidized lipids significantly decreases growth rate, feed intake and efficiency, immune function, and weight gain efficiency in pigs, especially in breeding animals, since the exposure occurs over long periods.

The ingestion of products resulting from the oxidative deterioration of fatty acids leads to irritability of the intestinal mucosa, diarrhea, and, in extreme cases, can result in liver degeneration and cell death. DeRouchey et al. (2004) observed reduced growth rates in pigs that are fed rancid white grease. Ringseis et al. (2007) reported that feeding oxidized sunflower oil increased oxidative stress markers in the small intestine of pigs, while Boler et al. (2012) reported that feeding pigs oxidized corn oil reduced growth performance (Table 1). Lu et al. (2014a) reported signs of liver damage in pigs subjected to dietary oxidative stress, increasing plasma bilirubin content, and enlarged liver size.

| Variable               | Corn oil quality × AOX inclusion <sup>1</sup> |                      |                         |                         | SEM  | P-value |      |           |
|------------------------|-----------------------------------------------|----------------------|-------------------------|-------------------------|------|---------|------|-----------|
|                        | Fresh – AOX<br>n = 8                          | Fresh + AOX<br>n = 8 | Oxidized –<br>AOX n = 8 | Oxidized +<br>AOX n = 8 |      | Oil     | AOX  | Oil × AOX |
| Initial BW, kg         | 80.2                                          | 80.3                 | 80.3                    | 80.2                    | 1.33 | 0.70    | 1.00 | 0.60      |
| Final study BW, kg     | 139.6                                         | 140.2                | 134.8                   | 137.2                   | 2.19 | 0.03    | 0.40 | 0.61      |
| Performance 0 to 28 d  |                                               |                      |                         |                         |      |         |      |           |
| ADG, kg                | 0.90                                          | 0.94                 | 0.80                    | 0.88                    | 0.05 | 0.12    | 0.20 | 0.67      |
| ADFI, kg               | 2.66                                          | 2.86                 | 2.55                    | 2.72                    | 0.13 | 0.26    | 0.10 | 0.89      |
| G:F, kg                | 0.32                                          | 0.33                 | 0.30                    | 0.33                    | 0.01 | 0.46    | 0.07 | 0.30      |
| Performance 28 to 56 d |                                               |                      |                         |                         |      |         |      |           |
| ADG, kg                | 1.23                                          | 1.20                 | 1.15                    | 1.15                    | 0.04 | 0.12    | 0.78 | 0.75      |
| ADFI, kg               | 3.27                                          | 3.22                 | 3.16                    | 3.17                    | 0.05 | 0.03    | 0.85 | 0.38      |
| G:F, kg                | 0.39                                          | 0.38                 | 0.39                    | 0.37                    | 0.01 | 0.58    | 0.21 | 0.78      |
| Performance 0 to 56d   |                                               |                      |                         |                         |      |         |      |           |
| ADG, kg                | 1.06                                          | 1.07                 | 0.97                    | 1.02                    | 0.03 | 0.03    | 0.37 | 0.57      |
| ADFI, kg               | 2.96                                          | 3.04                 | 2.79                    | 2.91                    | 0.08 | 0.04    | 0.14 | 0.77      |
| G:F, kg                | 0.36                                          | 0.35                 | 0.35                    | 0.35                    | 0.01 | 0.21    | 0.67 | 0.60      |

<sup>1</sup>AOX = antioxidant. Fresh –AOX diets included fresh corn oil without antioxidant; fresh +AOX diets included fresh corn oil +AOX; oxidized –AOX diets included oxidized corn oil without antioxidant; oxidized +AOX diets contained oxidized corn oil with antioxidant.

Table 1. Effects of dietary corn oil quality and antioxidant inclusion on barrow performance (Source: Boler et al., 2012)

There are some theories as to why oxidized feed causes such effects. According to Dibner et al. (1996), vitamins and polyunsaturated fatty acids deteriorate in the absence of antioxidants, and oxidized fats and their byproducts can negatively affect cells, resulting in changes in membrane permeability, viscosity, secretory activity, and membrane-bound enzyme activity. These primary effects lead to observable systemic effects. In order to prevent these damaging consequences, antioxidants have become a widely used alternative.

## The power of antioxidants

Chemical antioxidants (Table 2) are added to animal feeds to delay fat and vitamin oxidation, which keeps the diet palatable and helps prolong the feed's shelf life, ultimately maintaining the quality of the ingredients (Jacela et al., 2010). They prevent the binding of oxygen to free radicals. Dietary antioxidants have also been used in several species of animals to replace vitamin E, which is known for its antioxidant powers. Antioxidants are highly applicable in warm climates, when high levels of fat are added to the diet,

and in areas where byproducts high in unsaturated fats are commonly used.

| Ethoxyquin                                            | BHA                                                                    | BHT                                                                                     | TBHQ                             | PG                                                                  |
|-------------------------------------------------------|------------------------------------------------------------------------|-----------------------------------------------------------------------------------------|----------------------------------|---------------------------------------------------------------------|
| 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline         | Butylated hydroxyanisole                                               | Butylated hydroxytoluene                                                                | Tert-butylhydroquinone           | Propyl gallate                                                      |
| The most efficacious, followed closely by BHT and BHA | Effective in animal fats and relatively ineffective in vegetable oils. | Effective in animal fats, relatively less effective in vegetable oils (better than BHA) | More effective in vegetable oils | Works synergistically with other natural and synthetic antioxidants |

Table 2. Commonly used chemical antioxidants

Lu et al. (2014b) studied the effects of dietary supplementation with a blend of antioxidants (ethoxyquin and propyl gallate) on carcass characteristics, meat quality, and fatty acid profile in finishing pigs fed a diet high in oxidants. They reported that the inclusion of antioxidants minimized the effects of the high oxidant diet. The treatments including antioxidants, whether combined with vitamin E or not, had positive results in carcass weight, back fat, loin characteristics, and extractable lipid percentage.

Fernandez-Duenas (2009) studied the use of antioxidants in feed containing fresh or oxidized corn oil and its effects on animal performance, the oxidative status of tissues, meat quality, shelf life, and the antioxidant activity of skeletal muscle of finishing pigs. They reported that barrows fed with diets with the antioxidant blend showed increased feed efficiency. Orengo et al. (2021) showed that feeds protected with antioxidants could compensate for low vitamin E supply with regard to growth performance in the starter phase. Hung et al., 2017 theorized that the impacts on growth performance are likely related to the lack of adequate antioxidant capacity of the diet and oxidative stress status.

As literature and application results show, the use of antioxidants in pig feed is crucial to minimize adverse effects from oxidized feed and allow the animals to express their full performance potential.

## SANTOQUIN: preserving feed quality

From a practical standpoint, swine producers must consider some criteria for selecting a good antioxidant, which must preserve feed components, be nontoxic for humans and pigs, show effectiveness at very low concentrations, and be economically sustainable.

Considering those major characteristics, EW Nutrition offers a range of antioxidant solutions for the preservation of feed ingredients and feeds for poultry and swine through their SANTOQUIN product line. Santoquin is a feed preservative that protects supplemental fats, oils, meals, and vitamin premixes and protect feed from oxidation. Santoquin provides unsurpassed protection from oxidative rancidity, and it has proven effects against oxidation in feeds (Figure 1) ensuring the prolonged shelf life of feeds, especially during suboptimal storage conditions, such as those with high environmental temperature and elevated levels of moisture.

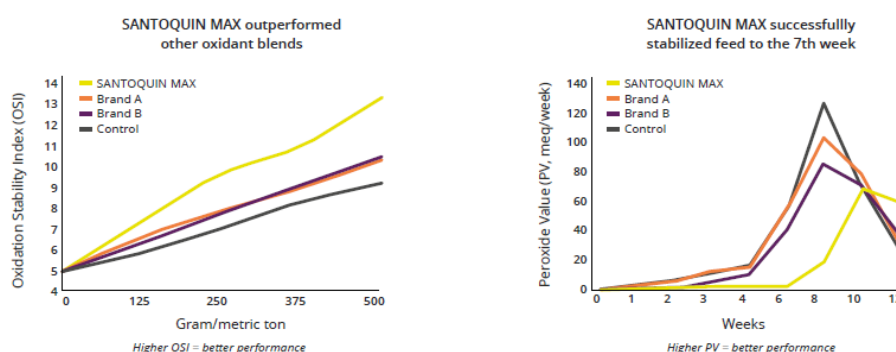


Figure 1. Antioxidant efficacy and competitiveness. SANTOQUIN MAX feed preservative is a proprietary antioxidant blend that effectively prolongs the shelf life of feed and feed ingredients by reducing oxidation rate.

Studies have been conducted to show the beneficial effects of Santoquin. Ethoxyquin, contained in Santoquin, has been used in the swine industry for over five decades and has been shown to improve growth performance and markers of oxidative status in pigs (Dibner et al., 1996). Ethoxyquin is also known for being the most efficacious and cost-effective antioxidant. Lu et al. (2014b) showed that the addition of an antioxidant blend (ethoxyquin and propyl gallate) protected pigs fed with a high-oxidant diet from oxidative stress more efficiently than vitamin E supplementation (Figure 2).

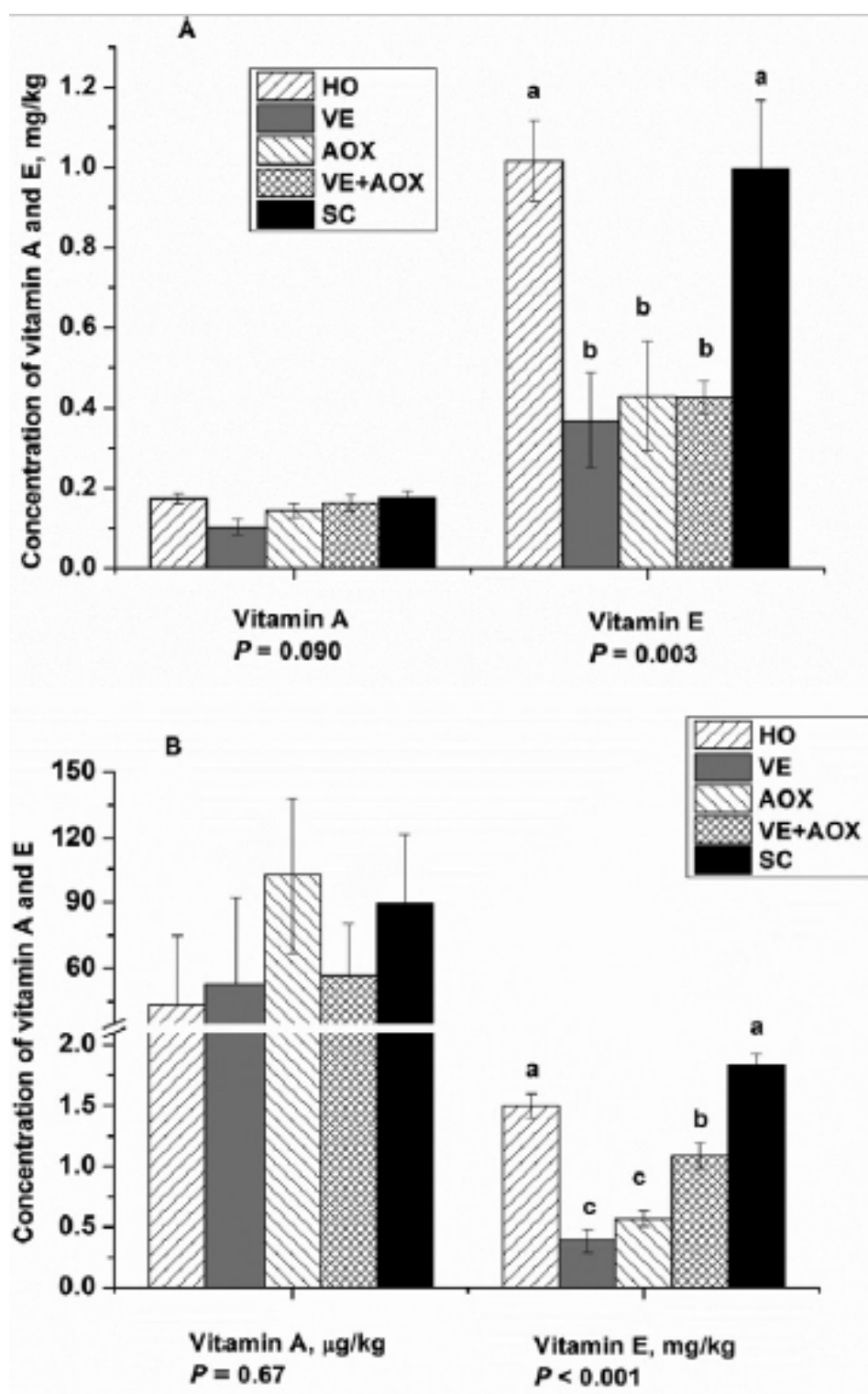


Figure 2. Concentrations of vitamins A and E across treatments in the plasma (A) and muscle (B). HO: high oxidant diet containing 5% oxidized soybean oil (peroxide value at approximately 180 mEq/kg of oil, 9 mEq/kg in the diet) and 10% of a PUFA source (providing approximately 55.57% of crude fat that contains docosahexaenoic acid [DHA] at 36.75%, and 2.05% DHA in the diet); VE: the HO diet with 11 IU/kg of added vitamin E; AOX: the HO diet with an antioxidant blend (ethoxyquin and propyl gallate, 135 mg/kg); VE+AOX: the

HO diet with both vitamin E and antioxidant blend; SC: a standard corn-soy control diet with nonoxidized oil and no PUFA source. The HO pigs were switched to the SC diet after day 82 as an intervention for poor health and performance. The samples came from two pigs from each pen. The VE treatment lost 1 replicate during the feeding phase and transportation period (n = 4), while in other treatments, n = 5. (Source: Lu et al., 2014b)

## Conclusion

The negative effects of oxidation in pig feed can result in diets with lower biological energy value. To avoid that, antioxidants help maintain intestinal health, ensure a safe food intake, preserve the ingredients and resources used in pig production. Overall, antioxidants help swine producers improve feed conversion and achieve more productive animals and lower mortality caused by toxicity. At the end of the day, the use of antioxidants is associated with better profitability.

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