Effect of ascorbic acid as a feed additive on indicators of the reproductive performance of Pulawska breed gilts

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Summary

The aim of the study was to determine the effect of vitamin C on indicators of gilts and sows reproductive value. The study was conducted using 64 gilts of the Pulawska breed. The experimental gilts received a supplement of vitamin C in an amount of 2.4 g daily throughout the whole experiment. Sixteen gilts from both control and experimental groups were mated in their third estrus. In order to determine the number of corpora lutea and the state of development of the reproductive organs, other gilts from the control and experimental groups were slaughtered after third estrus between the 5th and 10th day of the following estrous cycle. The results showed that gilts from the experimental group were characterized by higher potential fertility, as well as reared more piglets per litter. In the body weight and backfat thickness of sows during the first reproduction cycle in the control and experimental groups were not statistically significantly different. The piglets in the experimental group had higher body weight in the 1st and 21st day of rearing. Larger contents of protein, immunoglobulins, and vitamin C were recorded in the colostrum and milk from experimental sows.

Keywords: ascorbic acid, uterus, ovary, 17 β -estradiol, colostrum, milk, piglets, gilts

For many years, the opinion was prevalent that vitamin C should not be administered to swine because they are capable of synthesizing vitamins in sufficient quantities. Some reports revealed, however, that the risk of vitamin C deficiency in pigs is significantly greater than it was previously thought (1, 4, 12, 16, 39). An increased physical efficiency of an organism is noticeable after additional vitamin C supplementation, which is probably a consequence of stimulating the activity of hydroxylases involved in the synthesis of carnitine and improving the mitochondrial fatty acid oxidation (24, 27). The requirement for ascorbic acid increases rapidly in stressful situations, e.g. during high environmental stress and early postweaning (16). There was also a beneficial effect of vitamin C administration on the production and health effects on swine, especially atenatal sows and under conditions of strong stress (16). Vitamin C is an efficient enhancer of iron absorption (32). The use of vitamin C in the diet of female animals prevents the inflammation of

the mammary gland; it also stimulates the body's most important functions during the decrease of protein, vitamins and minerals levels during lactation (25). The stress responses on swine are highly expressed and therefore it is particularly appropriate to apply the protective effects of vitamin C (12, 20, 39, 45). Carmona-Garcia (3) reported that supplementation of sows with vitamin C for 1 week before farrowing had no effect on reproductive performance, whereas supplementation of vitamin C throughout gestation increased litter size. According to Sosnowska et al. (30) no clear effect on the rearing results of sows was found through the supplementation of vitamin E (200 mg/kg) and vitamin C (500 mg/kg diet). However, an advantageous post-effect of the vitamins fed to sows was shown: the addition of vitamin E together with vitamin C significantly reduced the body temperature of sows after farrowing and considerably reduced the number of sows culled after rearing. In the study Greer et al. (8) sows were fed 4 g of vitamin C per

day, however, a large daily dose of vitamin C was ineffective in countering seasonal infertility. In some studies, attempts have been made to use considerably higher doses of vitamins C in the feeding of pigs (21), than those recommended by the standards of many countries. Więckowski (43) recommended 2.0-2.5 g vitamin C daily per adult pig.

The aim of the study was to determine the effect of ascorbic acid added to a feed on indicators of reproductive performance of Pulawska breed gilts and sows, as well as the rearing of piglets.

Material and methods

The Local Ethics Committee, appointed by the University of Life Sciences in Lublin, approved the experiment described below (Nr 7/2012). The study was conducted in an individual breeding farm in the Lublin region. All pigs were maintained at the same environmental and feeding conditions. The diets were formulated according to the Nutrient Requirements of Swine (22). The experiment consisted of 64 Pulawska breed gilts with observed symptoms of the first estrus at the age of 6 months. Gilts were divided into two groups – 32 animals in each group. The experimental gilts received vitamin C in an amount of 2.4 g/animal/daily in a feed portion put in the empty trough prior to a mixture (Tab. 1) of complete feed according to the Polish Norm of Pig Nutrition (22). After the third estrus, 16 control gilts and 16 experimental sows were slaughtered between the 5th and 10th days of the following estrus cycle. Other sows in the control and experimental groups were mated during the third estrus. Mated experimental gilts were administered

with vitamin C in a quantity of 2.4 g per daily for the entire pregnancy (114 days) and lactation periods (35 days). Blood from the marginal ear vein was collected from experimental gilts during the tolerance reflex in the third estrus, and the concentration of 17 β -estradiol was determined by means of radioimmunoassay using RIA kit (Sorin Biomedica).

The reproductive organs were examined at slaughtered gilts. The assessment took into account the following factors: the weight of the uterus, ovarian weight (right and left), the length of the uterine horns (right and left), and the number of *corpora lutea*, whereas the mated gilts were weighed and fat thickness was measured using Renco camera PRE-ALERT – over the shoulder, behind the last rib, and at the cross (2 days before parturition, after parturition, and 35th day of lactation). The reproductive performance was also assessed according to the following: the number of live-born piglets, number of piglets reared up to the 21st day, weight of a piglet on the first day, and the piglet's weight on the 21st day. Colostrum and milk was collected from sows (after injecting 2 ml dose of oxytocin 10 j.m. Biowet Puławy Sp. z o.o.) immediately after parturition, and in the 7th and 14th days of lactation. The colostrum and milk samples were stored at about –20°C. The percentage of the main components in sow's colostrum and milk was determined applying Milko-Scan Camera 104. Immunoglobulins IgG were determined by means of radioimmunodiffusion technique (RID) using a kit Binding Site RID (Binding Site Limited). In addition, vitamin C content in colostrum and milk was also determined according to Roe and Kuether's method with Dabrowski and Hinterleitner modifications (7).

The obtained results were statistically processed calculating arithmetic mean ($\bar{x}$) and standard deviation (SD) values. The significance analysis of the differences between groups was performed using one-way variance analysis. All calculations were carried out using the Statistica Pl 6.0 software.

Tab. 1. Composition and nutritive value of mixtures

Dietary components	Gilts	Lactation sow
Barley (%)	79.75	15.10
Wheat (%)	–	37.22
Maize (%)	10.00	20.00
Soybean meal 46% (%)	8.00	20.00
Wheat bran (%)	–	5.00
Salt (%)	0.35	0.42
Dicalcium phosphate (%)	0.50	0.72
Limestone (%)	1.40	1.30
Premix L-lysine 20% (%)	–	0.24
Metabolizable energy MJ/kg	12.4	12.5
Dry weight (g · kg ⁻¹)	880	880
Crude protein (g · kg ⁻¹)	129	165
Crude fibre (g · kg ⁻¹)	48.2	42.3
Lysine (g · kg ⁻¹)	5.28	8.01
Methionina (g · kg ⁻¹)	2.04	2.58
Methionina + Cystine (g · kg ⁻¹)	4.57	5.65
Threonine (g · kg ⁻¹)	4.45	5.77
Tryptophan (g · kg ⁻¹)	1.44	1.92
Ca (g · kg ⁻¹)	7.04	7.66
P (g · kg ⁻¹)	4.61	5.64
Na (g · kg ⁻¹)	1.57	1.87

Results and discussion

The number of *corpora lutea* significantly increased by 0.81 in the experimental group. Vitamin C addition also resulted in an increased synthesis of 17 β -estradiol in the sow's organism by 2.68 pg/ml. Webb et al. (38) emphasized that among many environmental factors affecting the development of ovaries, dietary factors are the most important. Mahan and Vallet (18) indicated

Tab. 2. The reproduction organs and number of *corpora lutea* of gilts after the third estrus ($\bar{x} \pm SD$)

Parameters examined	Group	
	Control	Experimental
Uterus weight (g)	359.35 $\pm$ 42.10	374.67 $\pm$ 41.62
Right ovary weight (g)	4.38 $\pm$ 0.26	4.51 $\pm$ 0.25
Left ovary weight (g)	4.43 $\pm$ 0.30	4.62 $\pm$ 0.28
Length of right horn of uterus (cm)	55.86 $\pm$ 11.12	58.34 $\pm$ 11.37
Length of left horn of uterus (cm)	57.63 $\pm$ 10.82	59.71 $\pm$ 11.27
Number of <i>corpora lutea</i> (pcs.)	15.13 $\pm$ 1.26 ^a	15.94 $\pm$ 1.01 ^b
17 β -estradiol (pg/ml)	31.15 $\pm$ 3.76 ^a	33.83 $\pm$ 3.53 ^b

Explanations: a, b – values in rows with different letters differ significantly ($p \leq 0.05$)

the stimulatory effect of various vitamins and minerals added to a feed on the sexual cycle of sows. A rapid growth and development of young growing gilts in their deficient diet during large-scale production could additionally affect the effect of increased weight of gilt's reproductive organs, which was achieved due to the administration of vitamin C. Vitamin C supplementation caused the increased levels of 17β -estradiol which influenced the growth and development of reproductive organs (6, 40).

Some authors (29, 41) believe that backfat thickness above 16.0 mm is a prerequisite of good results in reproduction. Demonstrated lower body weight losses and reduced body fat losses during lactation suggest that the addition of vitamin C helped to maintain body weight and backfat thickness at a more stable level as compared to the control group. Lactating sows utilize reserves of the body accumulated in a form of subcutaneous and visceral fat, and when those reserves are depleted, sows intake energy from other tissues, which have a significant impact on body weight loss and

exhaustion of their organisms (28, 42). It was demonstrated that vitamin C alleviates the effects of stress in the body (5, 12, 45) and may indirectly contribute to the improvement of many indicators of sow's reproductive performance. It is likely that vitamin C also acts similarly to other organic acidifiers that along with lowering the pH, increase the activity of proteolytic enzymes and inhibit the growth of pathogenic gastrointestinal flora, thereby improving digestion, absorption, and retention of nutrients (19), and in consequence affect the improvement of piglets rearing results (11).

A significant difference was observed, between the control and the experimental group in the piglets reared on the 21st day of life (Tab. 4). More favorable results were obtained in the piglets reared to the 21st day of life. Sows receiving vitamin C reared more piglets per litter, which was associated with lower falls during rearing. Piglets from experimental sows had higher body weight on the 1st and 21st days ($P \leq 0.01$).

Pinelli-Saavedra et al. (20) reported that vitamin C had an effect on lymphocyte response in sows on day 103 of gestation and at 21 days post-farrowing. The improvement of piglet rearing presented in this study could be a result of indirect effects of vitamin C on the synthesis of L-carnitine (27). According to Ramanau et al. (24), L-carnitine has a positive influence on the growth and development of the fetus, resulting in the increase of birth weight and thus reducing the number of piglets with low birth weight and dead-born ones. Stress is a negative contributor to abortion, as well as it results in lower actual fertility and birth weight of a litter (24). Mahan and Saif (17) speculate that vitamin C may have a beneficial effect on the gastrointestinal tract of piglets. In the study by Sosnowska et al. (30), the supplementation of sows' feed with extra levels of vitamins E (500 mg/kg feed) and C (200 mg/kg feed) at the last stage of gestation and lactation had an influence on the increase in these vitamins in the serum of sows, their milk, and serum of piglets. According to these authors there was no effect of the increased addition of vitamins on the number of piglets that were born alive or dead, weight of the litter and weight of one piglet at birth and at weaning. Other researches

Tab. 3. Body weight and backfat thickness at sows during the first reproduction cycle ($\bar{x} \pm SD$)

Parameters examined	Group	
	Control	Experimental
Gross body weight gain during pregnancy (kg)	56.30 $\pm$ 4.55	58.62 $\pm$ 5.10
Parturition losses (kg)	17.81 $\pm$ 1.65	18.56 $\pm$ 1.71
Body weight loss up to 35 th day of lactation (kg)	18.56 $\pm$ 1.81	18.10 $\pm$ 1.66
Backfat thickness at mating (mm)	16.06 $\pm$ 0.98	16.69 $\pm$ 0.85
Backfat thickness 2 days prior to parturition (mm)	23.69 $\pm$ 1.75	24.75 $\pm$ 2.02
Backfat thickness on 35 th day of lactation (mm)	18.75 $\pm$ 1.49	19.94 $\pm$ 1.26

Tab. 4. Reproductive performance of sows ($\bar{x} \pm SD$)

Parameters examined	Group	
	Control	Experimental
Number of live-born piglets (animals)	10.44 $\pm$ 1.31	10.88 $\pm$ 1.15
Number of dead-born piglets (animals)	0.25 $\pm$ 0.45	0.19 $\pm$ 0.40
Number of piglets reared up to 21 st day (animals)	9.56 $\pm$ 1.03 ^a	10.31 $\pm$ 0.94 ^b
Weight of piglet on the 1 st day (kg)	1.30 $\pm$ 0.18 ^A	1.40 $\pm$ 0.17 ^B
Weight of piglet on the 21 st day (kg)	5.89 $\pm$ 0.41 ^A	6.14 $\pm$ 0.36 ^B
Piglet falls up to 21 st day (%)	8.43 $\pm$ -	5.24 $\pm$ -

Explanations: means in rows differ significantly: a, b at $p \leq 0.05$; A, B at $p \leq 0.01$

Tab. 5. Chemical composition of sow's colostrum and milk ($\bar{x} \pm SD$)

Parameters examined	After parturition		7 th day of lactation		14 th day of lactation	
	Control	Experimental	Control	Experimental	Control	Experimental
Protein (%)	12.80 $\pm$ 1.01 ^a	13.64 $\pm$ 1.15 ^b	5.47 $\pm$ 0.48	5.62 $\pm$ 0.51	5.25 $\pm$ 0.34	5.41 $\pm$ 0.41
Fat (%)	5.31 $\pm$ 0.47	5.40 $\pm$ 0.49	6.78 $\pm$ 0.61	6.89 $\pm$ 0.67	6.48 $\pm$ 0.53	6.59 $\pm$ 0.49
Lactose (%)	4.00 $\pm$ 0.31	3.91 $\pm$ 0.29	5.11 $\pm$ 0.37	5.04 $\pm$ 0.33	5.15 $\pm$ 0.28	5.07 $\pm$ 0.30
IgG (mg/ml)	148.25 $\pm$ 9.23 ^a	154.31 $\pm$ 7.67 ^b	3.15 $\pm$ 0.32	3.27 $\pm$ 0.38	2.03 $\pm$ 0.17	2.10 $\pm$ 0.19
Vitamin C (μ g/ml)	24.40 $\pm$ 2.47 ^A	31.30 $\pm$ 2.65 ^B	11.80 $\pm$ 0.99 ^A	15.29 $\pm$ 1.31 ^B	7.92 $\pm$ 0.68 ^A	9.46 $\pm$ 1.17 ^B

Explanations: as in Tab. 4

(21) applied 1 g to 10 g of vitamin C to sows from their artificial insemination to day 21 of the piglets' lives. Sow reproductive performance and piglet performance to weaning were not affected by the supplementation with vitamin C.

Sows treated with vitamin C were characterized with higher protein content in the colostrum and were statistically significant (Tab. 5). Considering the content of fat and lactose, these differences were not statistically significant. Similar dependencies were not observed for the milk of sows. The whey fraction of protein in colostrum contained albumin and immunoglobulins IgG, IgA, and IgM (2). Immunoglobulins IgG were the primary antibodies of colostrum. A prominent increase in the IgG level in colostrum of sows directly after parturition was recorded in the experimental group in comparison to the control one. On Wang et al. (37) study on the supplementation of δ -aminolevulinic acid (10 mg/kg of feed, as fed) with vitamin C (500 mg/kg of feed, as fed) resulted in improved hematological profiles (total protein, albumin, and IgG) in sows. The level of vitamin C in the experimental group (both in colostrum and milk) was higher as compared with the control group ($P \leq 0.01$). Other authors (15) reported that the concentration of vitamin C was the highest between the 18th and 36th h postpartum.

Changes occurring in the chemical composition, as well as the amount of milk produced by the sow, are the result of genetic (36) and environmental factors, and depend mainly on nutrition (10, 44). Kim and Easter (10) reported that complete satisfaction of the nutritional requirements during pregnancy and lactation is reflected in the remarkable increase in the content of protein, amino acids, and fat in the milk of sows, especially in the early stages of lactation. Protein contained in colostrum and milk is a component, which is a subject to the largest qualitative and quantitative changes during lactation (2, 34). Similar qualitative and quantitative changes of the basic components of colostrum and milk (fat, protein, lactose) with Pulawska breed sows were also recorded in the present study, both for the control and experimental group.

Vitamin C contained in the ration, as a catalyst of many processes in an organism, has a considerable effect on the synthesis of several compounds, including immunoglobulins (13) and is present in high concentrations in leukocytes (20). This vitamin increases the protein content in plasma, alters the albumin to globulin ratio (9, 14). Therefore, the increase of plasma proteins under the influence of vitamin C may be reflected as an increase in the increased levels of protein in colostrum and milk, as it was reported in this experiment. Vitamin C also affects the synthesis of L-carnitine (23, 27), which according to Ramanau et al. (24), increases the quantities of produced milk, while increasing the content of primary nutrients (fat, protein, lactose).

The effect of the increase in selected indicators of sows' reproductive performance observed in the pres-

ent study can be seen as an anti-stress effect of vitamin C present in increased amounts in colostrum and the milk of sows. On the other hand, on account of its effect on the stimulation of the immune system it affects the increase in the level of immunoglobulins in sow's colostrum and piglet's organism (after drinking milk with higher content of immunoglobulin and vitamin C, which has an impact on the synthesis of antibodies in the organism). Thus it contributes to elevated piglet's immunity to pathogens, and so improving their survival by facilitating their rapid growth and development.

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