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THE EFFECT OF NEUROTROPIC SUPPLEMENTS ON LACTOGENESIS IN FEMALE PIGS AND THE DEVELOPMENT OF THEIR OFFSPRING

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ABSTRACT: The aim of study was to evaluate the influence of biologically active additives on the hormonal status of lactating sows and the absolute growth of suckling piglets. Glutam 1M, nanoaquachelates of germanium (NPs-Ge) and Quatronan-Se (Cu-NPs, Se-NPs, Cr-NPs, Ge-NPs, Mn-NPs) have been administered orally (lat. per os) for animals in different percentage doses and schemes. Five animals groups have been formed by the method of analogues: control (20 ml physiological solution) and four experimental (group M: 18 mg/kg Glutam 1M; group G18: 5 µg/kg Ge-NP + 18 mg/kg Glutam 1M; group G9: 5 µg/kg Ge-NP + 9 mg/kg Glutam 1M; and group Q: Quatronan-Se (0.02 ml/kg) administered for 14 days). Results showed positive effects of the use of supplements on prolactin secretion. Administration of 5 mg/kg live weight NPs-Ge for sows G18 and G9 for 4 days before the farrowing increased the level of prolactin in the blood serum. On farrowing day, the hormone level in these groups was 14.6 ng/ml (G18) and 13.42 ng/ml (G9), while in K and M groups it was 12.02 and 9.94 ng/ml, respectively. On the day of weaning, the highest prolactin content has been observed in the G18 group as 14.9 ng/ml. Also, suckling piglets from this group have had the highest growth during the studied period. During the entire suckling period, the growth of piglets in the G18 group was 3.65 kg and was higher compared to the K (2.94 kg), M (3.58 kg), G9 (3.31 kg) and Q (3.44 kg) groups. It's suggested that the scheme introduction of Germanium (5 mg/kg) additives, 4 days before and 10 days after farrowing + Glutam 1M (18 mg/kg) 3 days after farrowing is the most effective (group G18) in means of growth performance.

Keywords: Biologically active drugs, Glutam 1M, Prolactin, Hormone, Sows.

INTRODUCTION

The hormonal profile of sows is one of the markers that indicate the general condition of the female body and its reproductive capacity (Soede et al., 2011). As one of the main signs of the reproductive capacity of sows, milk production largely determines the growth, development, and safety of piglets (Quesnel, 2011). The amount of milk produced primarily depends on the structure and size of the sow's mammary gland (Zhang et al., 2018). An intense increase in the size of the lacteous gland occurs in the final stages of pregnancy and during lactation (Grahofer and Plush, 2023).

The process of milk formation in the secretory cells of the mammary gland of pigs (lactogenesis), like other animal species, is controlled by hormones. Endocrine regulation of lactation involves adrenocorticotropic, somatotropic, thyroid-stimulating hormones, insulin, prolactin and oxytocin. Prolactin is the most important one (Kemp et al., 1978; Lacasse et al., 2011). Prolactin is involved in the biosynthesis of α-lactoalbumin, lactose, casein and directly affects the epithelial cells of the lacteous gland (Kemp et al., 1978; Iakubchak et al., 2010). According to Trokoz et al., (2014), Loisel et al. (2015) prolactin stimulates the growth of the lacteous gland and milk synthesis. It is also known that prolactin in milk enables the maturation of the neuroendocrine and immune systems of the newborn (Vlasenko, 2013).

It was found that during gestation, the prolactin content reaches a peak value at the time of farrowing (Garcia-Ispierto et al., 2009). After farrowing, prolactin secretion is supported by sucking and udder massage (Pilipchuk and Sheremeta a, 2016). It was also investigated and found that the level of prolactin in the blood of sows on the day after farrowing increases to 86.42-96.72 ng/ml against its concentration of 48.44-52.20 ng/ml on the 113th day of gestation (Saletskaya, 2008). Pilipchuk and Sheremeta (2016a) found that Glutam 1M affects the hypothalamic-pituitary system, which confirms the effect of this drug on increasing the content of prolactin in the serum of sows on the 4th day of the weaning period.

Therefore, further study of the effect of biologically active, environmentally friendly supplements on the hormonal status of sows' blood remains a timely area of research. The aim of the study was to investigate the effects of Germanium

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nanoaquachelate (Ge-NPs) Glutam 1M and Quatronan-Se [Cu (NPs), Se (NPs), Cr (NPs), Ge (NPs), Mn (NPs)] supplements and effective schemes of their administration on the body of lactating sows and the growth of suckling piglets.

MATERIALS AND METHODS

Experimental design

The experimental study was conducted in the experimental farm (Stepne Farm), which is located in the Poltava region (Ukraine) on sows of the large white breed, which were selected by the method of groups of analogues based on live weight, age, origin and farrowing, From the selected animals, 5 groups were formed - control K and four experimental group M, G18, G9, Q. There were 5 sows in each group. The experimental animals were inseminated with the sperm of a single sire boar. Therefore, the influence of parents on the results was levelled out.In previous studies, the doses of administration for each of the additives were determined separately. The most effective doses of Glutam 1M were 18 mg/kg and 9 mg/kg, and Germania - 5 mg/kg. Quatronan-Se was used in pigs for the first time, so the recommended dose of 0.02 mg/kg was used, which was used for cattle. This experiment investigates the most effective supplementation scheme.

Supplements (Table 1) to avoid stress in sows were administered orally according to the study scheme:

- Group K (control): physiological solution (20 ml) 4 days before farrowing and 10 days after;
- Group M: Glutam 1M (18 mg/kg of live weight) 3 days after farrowing;
- Group G18: Germanium nanoaquachelate (5 μg/kg) 4 days before and 10 days after farrowing + Glutam 1M (18 mg/kg) 3 days after farrowing.
- Group G9: Germanium nanoaquachelate (5 μg/kg) 4 days before and 10 days after farrowing + Glutam 1M (9 mg/kg) 3 days after farrowing.
- Group Q: Quatronan-Se (0.02 ml/kg) was administered 14 days, 4 days before and 10 days after farrowing. The composition of the supplements includes nanoparticles of trace elements Cu, Se, Cr, Ge, Mn in the form of carboxylates.

5 days before the expected date of farrowing, the sows have been transferred to a farrowing room.

Table 1 - The composition of supplements.

Supplements					
Germanium nanoaquachelate		Glutam 1M		Quatronan-Se	
Component	%	Component	%	Component	%
Germanium nanoaquachelate ($Ge_n^{2n-}(H_2O)_n$)	100	Monosodium glutamate ($C_5H_8NNaO_4$)	16	Cu (NPs)	19
		Sodium carbonate ($NaHCO_3$)	9	Se (NPs)	5
		Distilled water	75	Ge (NPs)	23
				Cr (NPs)	5.5
				Mn (NPs)	27.5 %
				Distilled water	20 %

NPs: nanoparticles.

Feeding

The sows were fed with complete ration compound feed, which was produced at the compound feed factory according to a special recipe. Pig rations were balanced in exchangeable energy, digestible protein, essential amino acids, minerals and vitamins. Before farrowing, the dry feed in the diet of sows was 3.3 kg per day, after farrowing – 6.3 kg per day (Barley: 25%, wheat: 28.5%; corn: 15%, sunflower meal: 4%, soybean meal: 15%, wheat bran: 5%, sunflower oil: 2.5%, premix - 5%).

Serum hormones concentrations

Blood collection from sows was performed on the day of farrowing, the 4th day of the suckling period, and the on day of weaning (Table 2). Blood was taken from the ear vein in the morning before feeding in dry sterile vacuum tubes with a capacity of 4 ml. The samples were settling at room temperature for 2-3 hours before clotting reaction to obtain blood serum. The resulting serum was centrifuged for 20 minutes at 1500 rpm, and then pipetted into microtubes (1.5 ml). Selected samples were frozen in liquid nitrogen for further analysis to determine the level of hormones in the sow's body. The content of prolactin, progesterone, testosterone and oestradiol in sows' blood was determined. The study was performed in the certified and accredited laboratory on the immunoassay analyser Immunochem-2100 using special reagents (LLC "Hema").

Table 2 - The scheme of blood sampling in experimental sows		
Group	**Blood collection days**	**Indicator**
Group K (Control)	On the farrowing day	Prolactin hormone
	On the 4th day after farrowing	
	On the 28th day after farrowing (weaning)	Prolactin, progesterone, testosterone estradiol hormones
Group M	On the farrowing day	Prolactin hormone
	On the 4th day after farrowing	
	On the 28th day after farrowing (weaning)	Prolactin, progesterone, testosterone estradiol hormones
Group G18	On the farrowing day	Prolactin hormone
	On the 4th day after farrowing	
	On the 28th day after farrowing (weaning)	Prolactin, progesterone, testosterone estradiol hormones
Group G9	On the farrowing day	Prolactin hormone
	On the 4th day after farrowing	
	On the 28th day after farrowing (weaning)	Prolactin, progesterone, testosterone estradiol hormones
Group Q	On the farrowing day	Prolactin hormone
	On the 4th day after farrowing	
	On the 28th day after farrowing (weaning)	Prolactin, progesterone, testosterone estradiol hormones

Statistical analysis

Statistical processing of the obtained results was performed using the programmable module "Data Analysis" in Microsoft Excel. The arithmetic mean (M) and its error (m) were determined. The level of statistical significance (P) was determined using the Student's t-test table. The results are considered statistically significant at $P<0.05$, $P<0.01$.

RESULTS AND DISCUSSION

Lactogenesis is initiated before farrowing by the peak of prolactin, which is induced by a decrease in the concentration of progesterone in sows' organisms. In addition, prolactin stimulates the growth of mammary glands and milk synthesis (Pilipchuk and Sheremeta, 2016a; Loisel et al., 2017).

In the serum of sows of the G18 and G9 groups after the farrowing there was a tendency to increase in the concentration of prolactin compared with the control and the M groups. The content of prolactin in the blood serum in groups G18 (14.6 ng/ml) and G9 (13.42 ng/ml), was higher compared with the K group (12.02 ng/ml) and the M group (9.94 ng/ml) (Table 3). The content of prolactin in the M group after the farrowing was lower by 2.08 ng/ml compared with the K group. A low level of prolactin in this group after the farrowing is not associated with the use of supplements because animals did not receive them before farrowing. Therefore, the indicators of the M group can be equated to the control data. The results of studies of the Q group (11.34 ng/ml) show that the concentration of prolactin in this group was higher compared to the M group, and lower, compared to the K (control).

Thus, it can be assumed that the introduction of Germanium and Quatronan-Se supplements in 4 days before the farrowing stimulates the synthesis of prolactin. A further experimental study showed a slight decrease in the content of prolactin in the serum of sows on the 4th day of the weaning period in the K (control), G18 and Q groups; and an increase in this indicator for animals of the M and G9 groups compared with data after the farrowing. Such changes can be explained by the fact that before the farrowing the secretion of this hormone increases significantly in response to estrogen, and after the farrowing its content is maintained during lactation by neuro-reflex way (Farmer, 2022).

Based on the report of Pilipchuk and Sheremet (2016 b), one can suppose that the additive Glutam 1M affects the hypothalamic-pituitary system, which confirms the effect of this supplements on increasing the content of prolactin in the serum of sows on the 4th day of the suckling period. In the M (10.8 ng/ml) and G9 (14.58 ng/ml) groups, the hormone concentration increased compared to the K (10.54 ng/ml) and compared to its content after the farrowing (M - 9.94 ng/ml; G9 -13.42 ng/ml). It was also found that this indicator in the G18 group -11.76 ng/ml was higher compared to the K, M and Q (10.54 ng/ml, 10.8 ng/ml, 7.76 ng/ml) groups (Table 3). Data on the concentration of prolactin in the blood of experimental sows on the day of weaning show that this indicator was the highest in the G18 group and was 14.9 ng/ml. While in the K- 12.8 ng/ml, M - 10.6 ng/ml, G9 -10.6 ng/ml and Q - 10.78 groups.

Taking into account the results of the effect of supplements on the induction of prolactin, and hence on milk secretion, we decided to investigate the growth of piglets obtained from sows of each group.

The analysis of research results regarding the growth (from 1 to 11 days) shows (Table 4) that during the 11 days of the suckling period it was significantly higher in piglets of groups M (1.72 kg), G18 (1.69 kg), G9 (1.69 kg) and Q (1.67 kg) compared to the control group (1.27 kg). It should be noted that in the M group during this period the growth in live weight of piglets was higher than for piglets of the G18, G9 and Q groups, but was within error. At the same time, between the 11th and 21st day of the suckling period, the growth for animals of the K group (1.53 kg) was lower compared to the M group (1.86 kg, P<0.05), G18 (1.96 kg), G9 (1.61 kg) and Q (1.75 kg). The growth dynamics of the animal of the G18 group was superior to the other experimental groups the growth of piglets of this group was 1.96 kg, and was higher than in the M, G9 and Q groups. Over the entire suckling period the growth of piglets of groups M, G18, G9 and Q was 3.58 kg, 3.65 kg, 3.31 kg and 3.44 kg, respectively, and was higher than that of K by 0.64 kg (P<0.001), 0.71 kg (P<0.001), 0.37 kg (P<0.05) and 0.5 kg (P<0.01), the difference was statistically significant. It should be noted that the growth of suckling piglets for the entire study period was the highest in the G18 group (3.65 kg).

From the results you can see that all the studied supplements have a positive effect on the growth rate of piglets. From the analysis of growth, which is shown in Table 3, and the content of prolactin in the blood of experimental sows (Table 2), it can be argued that the scheme of administration of Glutam 1M at a dose of 18 mg/kg, combined with germanium nanoaquachelate of after the farrowing is the most effective. As the concentration of prolactin was the highest in this group, this indicates that these supplements have a higher stimulating effect on the pituitary gland and, as a result, on milk secretion. The effectiveness of the application of this scheme is evidenced by the results of the growth in live weight of piglets, in the second experimental group they were the highest for the entire post-weaning period. Our results are confirmed by the results of Rezaei et al. (2022), who found that feeding a dietary supplement containing monosodium glutamate increases milk consumption by piglets by 14% and, accordingly, increases growth by 23-44%.

Also, to analyse the effect of the studied additives on the reproductive capacity of sows, the concentration of progesterone, testosterone and estradiol in the blood of sows on the day of piglet weaning (28 days after farrowing) was determined (Table 5). Testosterone levels in all study groups ranged from 1.11 to 1.77 ng/ml and corresponded to all physiological norms.

Table 3 - The content of prolactin in the blood of sows on different days of the suckling period, ng/ml (n=5)

Group \ Indicators M±m	After the farrowing	4-th day	Weaning
Group K (control)	12.02±0.97	10.54±2.09	12.8±0.98
Group M	9.94±1.45	10.8±1.79	10.6±1.77
Group G18	14.6±1.18[a]	11.76±2.31	14.9±0.12[a]
Group G9	13.42±1.63	14.58±2.19[b]	10.6±1.2[b]
Group Q	11.34±2.00	7.76±1.22[c]	10.78±2.18

[a]P<0.0 - compared with the M group; [b]P<0.01: compared with the G18 group; [c]P<0.05: compared with G9 group; M: arithmetic mean; m: standard error of means

Table 4 - Growth at different intervals of the sucking period, kg

Group \ Indicators M±m	For 11 day	Within 11 and 21 days	Whole suckling period
Group K (control)	1.27±0.097	1.53±0.122	2.94±0.140
Group M	1.72±0.064[b]	1.86±0.091[a]	3.58±0.114[b]
Group G18	1.69±0.091[a]	1.96±0.073[b]	3.65±0.131[b]
Group G9	1.69±0.091[a]	1.61±0.083[a]	3.31±0.107[a]
Group Q	1.67±0.057[b]	1.75±0.073[a]	3.44±0.103[b]

[a]P<0.05: compared with the K; [b]P<0.01: compared with the M group; M: arithmetic mean; m: standard error of means

Table 5 - Concentration of hormones in the blood of sows on the day of weaning and their subsequent reproductive capacity (n=5)

Groups \ Indicators M±m	Progesterone n/ml	Testosterone ng/ml	Estradiol pg/ml	First estrus after weaning (days)
Group K (control)	18.66±1.76	1.23±0.27	39.8±4.83	6.7±0.84
Group M	23.46±2.16	1.11±0.04	50.6±7.08	3.8±0.84
Group G18	21.48±1.97	1.74±0.26[a]	50.0±5.7	4.0±1.22
Group G9	23.34±4.87	1.56±0.16[a]	49.6±5.09	3.6±0.89
Group Q	21.56±4.16	1.77±0.10[b]	50.6±6.61	4.2±1.30

[a]P<0.05: compared with the K; [b]P<0.01: compared with the M group; M: arithmetic mean; m: standard error of means

It is known that the content and the ratio of sex hormones in the blood of females vary depending on their physiological condition. The concentration of progesterone and oestradiol in the serum characterizes the stage of the reproductive cycle of animals. The highest level of progesterone is observed during the corpus luteum phase and pregnancy, and the lowest level during the beginning of the follicular phase (Seba and Khomenko, 2017). In our studies, it was found that the lowest level of progesterone was in the K group as 18.66 ng/ml, and the highest in the M group – 23.46 ng/ml, the difference was 20.6%. In the G18, G9 and Q groups, the hormone concentration was at the level of 21.48, 23.34 and 21.56 ng/ml, which is by 13.1, 20.0 and 13.4% higher compared to control animals. Based on the fact that supplements have been used in the M, G18, G9, Q groups, and the level of progesterone the was higher compared to animals in the K group, it can be assumed that the studied supplements affect the synthesis of progesterone.

Analysis of the content of oestradiol in the serum of sows shows that the lowest level of this indicator, as well as progesterone, was in the K group, and was 39.8 pg/ml, while in the M, G18, G9, Q groups it was higher by 21.3; 20.4, 19.8 and 21.3%. The difference between the experimental groups was within 2%. It should be noted that the animals in the control group came into heat 6.7 days after weaning, while in the experimental groups it was 2.9 (M), 2.7 (G18), 3.1 (G9) and 2.5 (Q) days earlier.

The supplement Glutam 1M was created on the basis of glutamine amino acid, which belongs to the 116 neurotransmitter amino acids that stimulate the transmission of excitation in the synapses of the central nervous system (Mosharova et al., 2004). Studies by several scientists testify to the influence of glutamate on the endocrine cells of the adenohypophysis, thus it can induce the release of prolactin (Frederick et al., 2006). During usage of the supplement Glutam 1M, an increase in the prolactin level was observed in sows, and an increase in growth in piglets of these groups. Based on this, it can be assumed that the supplement, which includes monosodium glutamate, stimulates the pituitary gland to release an additional amount of prolactin into the blood, and due to prolactin, the secretion of milk increases and its consumption by piglets increases, which contributes to the increase in growth of in these groups compared to group K.

The results of the studies of the Q group show that the microelements included in the supplement do not affect the prolactin level; however, in this group the growth was 14.5% and 3.8% higher compared to groups K and M. Such indicators show a positive effect of microelement not only on the body of the sow, but also through milk on the body of suckling piglets. According to the research data of some authors, it is known that the administration of selenium in the form of chelated form to farrowing and suckling sows contributes to the increase of multiple fertility, litter weight on the day of farrowing, milk yield, and also increases the average growth and survival of suckling piglets (Usenko et al., 2019). In addition, the researchers found that adding Cu at a dose of 60 to 250 mg to sows before farrowing reduced piglet mortality and increased piglet weight due to increased milk production in the sow. According to their data, the higher average daily growth may be associated with the participation of copper in the regulation of mRNA expression of neuropeptide Y, which stimulates feed consumption by animals.

Pilipchuk and Sheremeta et al. (2016 a, b) established that the Glutam 1M supplement /component affects the hypothalamic-pituitary system in sows. In addition, glutamate affects the hypothalamus and, due to an increase in glutamatergic transmission through KiSS-1 peptide to GnRH neurons, stimulates the increase of antral follicles (Bezverkha et Al., 2019; Meza-Herrera et. al, 2020; Santos Soares et. al, 2023). The results of research showed that feeding Glutam 1M to sows of a large white breed causes a tendency to increase the concentration of progesterone and oestradiol in the blood, which contributes to the improvement of their reproductive capacity, namely: fertilization, multiple fertility, high fertility and preservation of piglets in the embryonic period increase (Pilipchuk and Sheremeta, 2016 a; Bezverkha et Al., 2019). According to the results of research, the oestradiol level was higher by 21.3% and 21.3%, progesterone by 20.6% and 20%, in the groups usage Glutam 1M at a dose of 18 mg per kg. In the Q group (Table 5), there is also an increase in these indicators compared to the control, which is probably related to the fact that trace elements such as copper, manganese, selenium, chromium included in the Quatronan-Se are directly related to the action of enzymes, hormones (Scott, 2005; Nicola et al., 2013). They are the part of vitamins, take an active part in the metabolism of nucleic acids, protein synthesis, tissue differentiation and growth, and reproductive capacity (Hayirli, 2005), Manganese takes a direct part in the synthesis of cholesterol, which in turn affects the formation of sex hormones, including progesterone and estrogen (Jan et al., 2022). Cr plays an important role in the production of insulin, which affects the synthesis of progesterone, LH (luteinizing hormone) and stimulates the growth of follicles and ovulation (Kafilzadeh et al., 2012; Jan et al., 2022).

The analysis of the subsequent reproductive function shows that the animals of the experimental groups came into heat on average 3-4 days after weaning, while the sows of the control group came into heat on the 6-7th day (Table 5). It is known from the scientific literature that at the end of the follicular phase, under the influence of luteinizing hormone, progesterone is secreted by the ovaries. Ovulation occurs against the background of a high level of oestradiol and the release of progesterone (Rosca et al., 2017; Langendijk, 2021). These data confirm the results of present research, from which we can conclude that the studied supplements and their administration schemes have a positive effect on the further reproductive capacity of sows.

CONCLUSION

It was found that feeding sows (before and after farrowing) with nanoaquachelate germanium, Glutam 1M and Quatronan-Se supplements have a positive effect on the synthesis of prolactin in sows and the growth of suckling piglets. The use of the above supplements helps increase the sows' reproductive capacity.

- The highest level of prolactin in the blood was observed in the sows of G18 group as 14.6 ng/ml after farrowing and 14.9 ng/ml – on the day of weaning piglets, respectively 21.5 and 16.4% more compared to control animals.
- During the entire pre-weaning period, the piglets of group G18 had a growth in live weight of 24.1; 1.9; 9.3, and 5.7% higher than the analogues of the K, M, G9 (P<0.05), and Q groups.
- Sows of the M, G18, G9, Q groups went back into heat on average 3-4 days after weaning piglets, and animals of the K group, 6-7 days.

It can be suggested that the proposed regimen for sows administrating with Glutam 1M at a dose of 18 mg/kg, with nanoaquachelate germanium at a dose of 5 mg/kg (study group G18) is the most effective.

DECLARATIONS

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Ethical regulation

When conducting the study, the rules of handling animals were followed in accordance with the European Convention "On the Protection of Vertebrates. Animals Used for Research and Other Scientific Purposes" (1986). The protocol for taking blood from sows was agreed with the Bioethical Commission of the National University of Life and Environmental Sciences of Ukraine (No. 002/2023).

Data availability

The datasets used during the current study available from the corresponding author on reasonable request.

Authors' contribution

Inna Kurbatova, Ihor Holovetskyi, and Inna Kepkalo helped in the planning and design of the study. Maryna Khomenko, Seba Mykola, Ruban Serhiy, Bogdanova Nataliya participated in the conducted research and processing of the received data. Mykola Seba and Maryna Khomenko revised the manuscript critically. All authors have read and approved the published version of the manuscript.

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Competing interests

The authors have not declared any conflict of interests.

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