



Dietary effects of drone larvae homogenate on the homeostatic constants and the reproductive capacity of Large White gilts

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Abstract

Background: With modern requirements for housing agricultural animals particularly pigs, the manifestation of normal physiological functions significantly changes, especially reproductive one, which is manifested in an insufficiently expressed sexual cycle, reduced resistance due to technological stress. There is a need to develop effective reproductive biotechnologies in pig using organic stimulants of entomological origin, among which a significant place is given to the homogenate of drone larvae (HDL). The aim of the study

was to determine the dietary effects of HDL on the formation of prooxidant-antioxidant homeostasis in Large White (LW) gilts, as well as to optimize reproductive function during experimental feeding of HDL.

Methods: In the study, gilts representing Large White breed were investigated. A total of 20 gilts representing two groups. The first feeding group (n=10) of LW gilts was fed with the control normal diet, and the second feeding group (n=10) of LW gilts was fed with the supplement diet i.e., the biological additive of homogenate drone larvae in quantity of 0.5g daily. To assess prooxidant-antioxidant homeostasis in pigs, blood samples were taken from the anterior vena cava into the diestrus phase i.e., 11 days from the beginning of the second cycle after the determination of the immobility reflex and the estrus phase i.e., 24 days after the start of estrus after the determination of the immobility reflex. Intracervical artificial insemination of gilts was performed during 24 and 36 hours after the determination of the immobility reflex, and depending on the manifestation of signs of estrus.

Results: Study found an intensification of peroxidation processes during the period of puberty in LW gilts, which was manifested in an increase in the content of diene conjugates on the 180th and 210th days of development by 63.6% and 44.1%, respectively. This is confirmed by a significant increase in the number of secondary products of peroxidation – TBA-active complexes in these periods of their development. Study revealed that during puberty of gilts the antioxidant defense system was the most labile from 6 to 8 months of age, where the variability of PAG components was significant in terms of increased activity of superoxide dismutase ($p < 0.01$) and catalase ($p < 0.05$), as well as the amount of reduced glutathione. Moreover, additional feeding of HDL contributed to the probable slowing down of peroxidation processes in blood, which was confirmed by the lower content of diene conjugates ($p < 0.05-0.01$) and TBA-active complexes ($p < 0.01-0.001$).

Conclusions: Study concluded that experimental supplementary feeding of HDL diet to LW gilts stimulated the reproductive function during their puberty by reducing the time to the first estrous cycle for artificial insemination, thus improved the fertility in LW gilts.

Keywords: homeostasis; Large-White; gilts; sexual cycles; enzymes; peroxidation; homogenate of drone larvae.

Introduction

A large number of anthropogenic factors provoke chronic stress and metabolic disorders in sows, which is accompanied by a decrease in the

manifestation of sexual cycles [1]. The use of various biologically active substances to synchronize estrus and the onset of farrowing is the best method to reduce the cost of insemination and receiving offspring. In today's conditions, the leading role in the nutrition and normalization of sexual function in pigs belongs to the use of complex biologically active additives, among which an important nutrient diet of homogenate of drone larvae (HDL), also known as drone brood homogenate (DBH) [2–4]. Previous study showed that feeding of HDL to boars alters prooxidant-antioxidant homeostasis in the direction of slowing down the course of peroxidation processes, which is accompanied by an improvement in sperm quality and sperm fertility [5]. The positive effect of HDL on the body of pigs causes an increase in testicular weight, epididymis and sperm quality, as well as increasing the reproductive capacity of sows [6]. This is due to the presence of vitamins, limiting amino acids and hormones (testosterone, progesterone, prolactin, estradiol) in HDL, which do not significantly change the endocrine profile in animals [6]. It is proved that the processes of fertilization, implantation, placentation and growth and development of embryos occur with the direct participation of hormones and free radicals, the amount of which is controlled by the state of prooxidant-antioxidant homeostasis (PAG) [7–10]. Due to this leading role of active forms of oxygen in the processes of reproduction of pigs, it is important to study the features and patterns of formation of prooxidant-antioxidant homeostasis in gilts, to develop ways to correct it, which can be the basis for methods to regulate their growth and development and increase reproductive function of pigs. Our study aimed to determine the dietary effects of HDL on the formation of prooxidant-antioxidant homeostasis in gilts, and to optimize reproductive function during experimental feeding of HDL.

Materials and Methods

The experiments were carried out at the Institute of Pig Breeding and Agricultural Production of NAAS. In the study, a total of 20 LW gilts

were investigated. The experimental design was comprised of two feeding groups. The first feeding group (n=10) of LW gilts was fed with the control normal diet, and the second feeding group (n=10) of LW gilts was fed with the supplement diet i.e., the biological additive of homogenate drone larvae in quantity of 0.5g daily. To assess prooxidant-antioxidant homeostasis in pigs, blood samples were taken from the anterior vena cava into the diestrus phase i.e., 11 days from the beginning of the second cycle after the determination of the immobility reflex and the estrus phase i.e., 24 days after the start of estrus after the determination of the immobility reflex. Intracervical artificial insemination of gilts was performed during 24 and 36 hours after the determination of the immobility reflex, and depending on the manifestation of signs of estrus [11]. The evaluation of reproductive qualities of LW gilts was performed on their fertilization, fertility, high fertility and weight of litter at birth and weaning at 28 days.

The blood samples of LW gilts were collected to determine the indexes of the state of PAG. To assess the level of peroxide oxidation, the concentration of diene conjugates – spectrophotometrically [12] and TBA-active complexes (aldehydes and ketones) photoelectrocolorimetrically [13] were determined. The level of antioxidant protection was determined by the activity of superoxide dismutase (SOD) photometrically by its ability to inhibit the oxidation of adrenaline in a biological sample. Inhibition of 50% of the rate of adrenaline autooxidation was taken as a unit of activity [14]; catalase activity (CT) according to the method using vanadium-molybdate reaction [15], the content of the reduced form of glutathione-photoelectrocolorimetrically with Elman's reagent [12]; concentration of ascorbic and dehydroascorbic acids for the number of ozones formed [13]. The resulting digital material was statistically processed using the Statistics for Windows XP. Student's T test to compare the investigated parameters and their intergroup differences, and the results were considered significant at different levels of $p < 0.05$, $p < 0.01$, and $p < 0.001$.

Results

Feeding gilts with biologically active HDL supplement significantly inhibited peroxidation processes (Table 1). This is confirmed by the probably lower concentration of new conjugates in LW gilts of the second experimental group relative to the control on 180th – 29.9% ($p < 0.001$), 210th – 47.3% ($p < 0.01$) and 240th day – 53.3% ($p < 0.001$). The dietary effect of this HDL additive was reduced the number of TBA-active complexes by 31.6% ($p < 0.01$) on 150th, 38.8% ($p < 0.001$) on 180th, 33.3% ($p < 0.001$) on 210th, 30.6% ($p < 0.001$) on 240th and 47.5% on 270th days of development.

It is important to note that the level of the antioxidant defense system changed significantly during puberty (Table 2). Thus, the activity of superoxide dismutase in the blood serum of investigated LW gilts significantly increased by 32.9% ($p < 0.01$) to the maximum values on the 180th day of life with a subsequent decrease. The maximum functional activity of catalase ($p < 0.05$) was detected when the animals reached 210th days of age. The probable predominance of ascorbic acid content in the experimental group relative to the control group was determined as 18.0% ($p < 0.001$) on the 180th day and 22.6% ($p < 0.001$) on the 270th day of development. In the concentration of its oxidized form significantly increased in the first control group on the 210th day by 14.4% ($p < 0.01$) and in the second experimental group on the 240th by 14.8% ($p < 0.001$) and on the 210th by 29.8% ($p < 0.01$) relative to the 120th day of life. It should be noted that during all research periods there was a predominance of the amount of oxidized form over the reduced, where the maximum difference was found in the first group by 38.8% and the second group by 18.0%.

Table 1. Dietary effects of HDL on the intensity of peroxidation processes in blood of LW gilts during puberty

Indexes	Feeding groups	Age (in month)							
		4	5	6	7	8	9		
Peroxide resistance of erythrocytes, %	1	14,2±1,36	12,3±1,4**	15,13±0,25	13,86±1,2	11,28±0,46	9,3±1,35		
	2	12,11±1,20***	12,8±2,05	13,4±0,83*	10,30±1,16**	8,22±0,40	7,13±0,93'		
Diene conjugates, μmol/l	1	2,29±0,44	3,05±0,30*	3,74±0,27*	3,30±0,18	3,0±0,40**	2,96±0,25		
	2	1,85±0,31	2,60±0,51	2,88±0,20**	2,24±0,30**	2,09±0,16'	1,93±0,18***		
The content of TBA-active complexes, μmol/l	1	34,85±6,94	39,66±3,34	46,38±1,06	40,59±4,08	37,38±1,31	38,97±4,43		
	2	31,39±6,30	30,13±4,22**	33,41±0,67***	30,43±4,34***	28,62±0,73***	26,41±3,22		
The content of TBA-active complexes after incubation, μmol/l	1	37,12±7,11	46,5±1,72	51,17±1,53	48,62±3,51	39,26±0,65	40,6±3,12		
	2	36,2±8,15	38,41±6,37	39,14±0,73***	33,12±5,14**	31,4±1,69***	30,01±3,43***		

Significant at: * - p < 0.05, ** - p < 0.01, *** - p < 0.001 compared to the first group. # - p < 0.05, ## - p < 0.01, ### - p < 0.001 compared to 4 months of life of pigs.

Table 2. The dietary effect of HDL on the state of antioxidant protection in blood of LW gilts during puberty

Indexes	Feeding Groups	Age (in month)							
		4	5	6	7	8	9		
Superoxide dismutase, USD/ml	1	0,65±0,10	0,72±0,11	0,97±0,08**	0,79±0,08*	0,63±0,05	0,50±0,14		
	2	0,67±0,11	0,58±0,12	0,65±0,05***	0,62±0,13*	0,53±0,03	0,45±0,08		
Catalase, H ₂ O ₂ /min/l	1	10,41±3,15	16,50±5,29	17,21±0,88	18,2±4,40*	14,38±0,52	12,8±2,33		
	2	9,18±1,89	12,41±2,61	14,80±0,86	10,33±3,25*	10,6±0,53**	8,82±2,76*		
Ascorbic acid, µmol/l	1	23,02±2,08	23,14±2,19	16,6±1,38**	18,0±3,48	20,8±1,16	23,34±3,11		
	2	22,02±3,06	22,3±2,54	20,6±1,20***	24,65±2,44*	25,2±1,8*	28,47±3,04***		
Dehydroascorbic acid, µmol/l	1	24,31±2,89	24,37±2,02	27,13±0,81	28,41±2,94**	25,4±1,77	29,37±3,37*		
	2	22,57±1,80	23,5±3,11	25,14±1,49	26,32±2,17	26,5±1,34	32,17±3,01		
Reduced glutathione, µmol/l	1	0,29±0,045	0,36±0,06	0,35±0,06	0,39±0,065	0,32±0,05	0,31±0,05		
	2	0,32±0,07	0,44±0,07*	0,47±0,11	0,44±0,08	0,39±0,03*	0,36±0,03		
β and pre-β lipoproteins, µmol/l	1	3,53±0,56	3,35±0,27	2,93±0,62	2,64±0,21	2,26±0,32	2,31±0,32		
	2	2,51±0,46	2,30±0,45	2,54±0,52	2,44±0,19	2,33±0,38	2,42±0,32		

Significant at: * - p < 0.05, ** - p < 0.01, *** - p < 0.001 compared to the first and second groups. ■ - p < 0.05, ■■ - p < 0.01, ■■■ - p < 0.001 compared with the 4 months of life of pigs.

These changes in the formation of PAG during puberty significantly affected the level of peroxide resistance of erythrocytes in the direction of its growth from 240th to 270th days of development. However, the use of HDL in gilts increased the resistance of erythrocytes to peroxide hemolysis, especially when they reach 7 and 8 months of age, where the difference between the control and experimental groups was 25.6% ($p < 0.01$) and 27.1%, respectively. The content of reduced glutathione significantly increased by 18.1% ($p < 0.05$) on the 150th, 25.5% on the 180th and 11.3% on the 210th day. Beta and pre-beta levels of lipoproteins did not change significantly during the experiment.

Results revealed that the biologically active supplement diet of HDL significantly affects the time of the first estrus, where the beginning of the first sexual cycle was set in the animals of the control group on the 162th day, when the gilts of the experimental group had on the 158th day of development (Table 3). A shorter duration of sexual cycles was recorded, which had 26 days from the first to the second estrus in the experimental group, while the control group had 28 days. The duration of the third cycle in the control was 23 days, the index of the experimental group was 22 days.

Table 3. Dietary effects of HDL on reproductive indexes of gilts

Reproductive indicators	Groups	
	Control	Experiment
I estrus, days	162,0±5,09	158,0±3,09
Duration of the II cycle, days	28,0±1,49	26,2±1,54
II estrus, days	190,0±3,82	184,0±2,26
Duration of the III cycle, days	23,0±1,69	22,1±1,91
III estrus, days	213,0±2,35	206,0±2,82
Number of piglets at birth, ch	12,0±0,94	12,5±0,20
Number of live piglets at birth, ch	11,1±0,50	11,3±0,17
Large-fertility, kg	1,3±0,10	1,33±0,15

Reproductive indicators	Groups	
	Control	Experiment
Litter weight at birth, kg	14,43±0,22	15,03±0,14
Quantity at weaning, ch	9,44±0,09	9,74±0,03
Saving,%	85,0±1,82	88,0±2,30
Litter weight at weaning at 28 days, kg	72,68±0,90	79,18±0,68

Study found that during the rearing of the animals of the control group, the onset of the third estrus was 213 days, and in the experimental group it was accounted for 206 days of development. The difference in the formation of sexual function in these animals was apparently due to the influence of the biologically active substance HDL.

Improving the state of PAG (under the influence of feed additive HDL) in blood of gilts during puberty reduced the duration of the first and third estrus by 2.5 and 4 days, respectively, which helped to stabilize the duration of the sexual cycle. There was a tendency to improve the reproductive qualities of gilts receiving this supplement, where the number of live piglets at birth was greater by 0.2 heads, the weight of the litter at birth by 600 g, the weight of the nest at weaning by 8.5 kg.

Discussion

Study results revealed that additional feeding of HDL with food has a corrective effect on the endocrine and circulatory systems, resulting in increased levels of thyroxine, triiodothyronine, cortisol, resistance and reproductive capacity of animals [5]. It is proved that rearing pigs on improved complete diets enriched with biologically active components (most of which are components of HDL) provides more biologically complete offspring, significantly reducing the percentage of culling and significantly increases the efficiency of the selection process [16]. A recent

study of Usenko [17] proved that depending on the periods of puberty of gilts and the reproductive cycle of sows, the state of prooxidant-antioxidant homeostasis changes, and its changes ensure the normal course of fertilization, implantation and placentation of embryos. [17]. However, the successful implementation of these measures requires a stable function of the neurohumoral regulatory system. That is why the correction and synchronization of the reproduction function is so important.

In our study, we confirmed the significant effect of biologically active substances on the reproductive capacity of pigs, However, the leading role in the nutrition of pigs belongs to the use of complex biologically active additives, of HDL, where its action was due to the presence of vitamins β -carotene, α tocopherol, vitamins B1, B2, B3, B4, B5 and B6), limiting amino acids (lysine, methionine, arginine, trionine) and hormones (testosterone, progesterone, prolactin, estradiol) [18–19]. Thus, in gilts during puberty, the course of peroxidation processes changes significantly, reaching maximum values when they reach 6-8 months of age. At the same time, additional feeding of HDL significantly slows down the course of peroxidation processes, which was accompanied by an increase in the level of antioxidant protection. Changes in PAG in blood serum of LW gilts that occur when HDL included in the diet had a positive effect on their reproductive indexes, which was manifested in a reduction in the time of their economic maturity, increased fertility, and weight of piglets at weaning.

Conclusions

Study proved that during puberty of LW gilts there is an intensive increase in the content of diene conjugates in the 6th and the 7th months of development. This may be due to a significant increase in the number of secondary products of peroxidation – TBA-active complexes. The dietary effects of HDL was determined that during puberty of gilts the antioxidant defense system was most responsive at 180 to 240 days of age,

where the variability of PAG components was higher than the increased state of superoxide dismutase ($p < 0.01$) and catalase ($p < 0.05$) and the content of reduced glutathione. The gradual introduction of HDL into the diet significantly reduced the content of diene conjugates ($p < 0.05-0.01$) and TBA-active complexes ($p < 0.01-0.001$) while inhibiting the processes of peroxidation in blood.

Study confirmed that HDL in the diet of LW gilts has a positive effect and corrects the formation of reproductive function. The formation of the first sexual cycle was accelerated, the time of manifestation of sexual cycles and the beginning of the third estrus was rationalized, the fertility of young pigs and the weight of the litter at birth and weaning increased significantly. However, subsequent studies require the development of options for control and influence on the course of gestation in sows and piglets based on the correction of their nutrition under the influence of organically formed biologically active additives.

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