ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXVII (2) SECTIO EE 2009

Department of Breeding and Pig Production, University of Life Sciences in Lublin,
Akademicka 13, 20-950 Lublin, Poland,
e-mail: jerzy.lechowski@onet.eu

JERZY LECHOWSKI

Effect of vitamin C on semen quality of duroc breed boars and their crossbreds with hampshire and pietrain

Wpływ witaminy C na jakość nasienia knurów rasy duroc i ich mieszańców z rasą hampshire oraz pietrain

Summary. The boars of duroc breed and duroc x hampshire and duroc x pietrain crossbreds received vit. C at a dose of 2.5 and 3.6 g/animal/day for 30 days. Prior to this vitamin administration, ejaculates from males were collected to constitute the control (K), while the experimental ejaculates (D) were recollected from the boars after vitamin C supply. The present research has revealed an increased semen volume as well as a growth of total ejaculate sperm count under study and their concentration with concurrent elevation of percentage of progressively motile sperm cell, insemination dose number and spermatozoon count per dose. The rising dose of vitamin C administered to boars implied some changes in the aforementioned parameters. Alkaline phosphatase activity elevated by vitamin C supply has improved the percentage of progressive motility spermatozoa. Besides, vitamin C increased pH value in boar semen.

Key words: vitamin C, semen, boar

INTRODUCTION

There has been ongoing research aiming at improvement of boar semen parameters all over the world. Quality of boar semen depends on several genetic factors [Kondracki *et al*. 2003, Kozdrowski and Dubiel 2004b] and a boar breed proves to critically [Wysokińska and Kondracki 2000] affect both, most of semen traits and environmental factors [Trudeau and Sanford 1986, Kondracki *et al*. 2002, Kozdrowski 2004]. As for the environmental conditions, the rearing conditions have a key role, whereas suitable nutrition and animal age are considered vital for good quality of boar semen [Kondracki *et al*. 2000, 2004, 2005]. Spermatogenesis is influenced adversely by some other environmental factors, like pesticides, exogenous female hormones and heavy metals, whereas beneficial impact is exerted by dietary supplementation with carnitine, arginine, sele-

nium, vitamin E as well as vit. B_{12}, B_6 and PP that increase spermatozoon number and improve their motility [Rebouche 1991; Jacyno *et al*. 2003; Kozink *et al*. 2004]. Antioxidants like, vit. C, vit. E, glutathione and coenzyme Q_{10} appeared to be effective for infertility treatment [Sindair 2000].

The objective of the present research was to determine the effect of vitamin C on some physical and chemical characteristics of ejaculate obtained from boars of duroc breed and duroc x hampshire and duroc x pietrain crosses.

MATERIAL AND METHODS

The boars of duroc breed and duroc × Hampshire and duroc × pietrain crossbreds aged approximately 20 months housed in the Station for Sow Insemination in Kraśnik, received vitamin C at a dose of 2.5 (D_1 – experimental group) and 3.6 g/unit/day (D_2) for 30 days during the winter-spring season. According to Więckowski [1980], a recommended daily vitamin C dosage for adult pig should reach 2.0–2.5 g. All the animals were fed commercial full ration diets without a vitamin C constituent which was established using the Roe and Kuether laboratory method, modified by Dabrowski and Hinterleitner [1989]. Each animal from the experimental group was housed in an individual pen to assure the administration of a required experimental dose of vitamin C which was supplied in a small feed stuff portion to the empty trough prior to full ration daily diet was provided. Before the vitamin C application, ejaculates were collected from boars to constitute the control (K). After the vitamin C application period, ejaculates were recollected from the males and made the experimental group – (D_1) or (D_2). From 18 juvenile boars (6 animals from each investigated group: D, D × H, D x P), ejaculates were collected every fourth day (a total of 270 ejaculates). Fifteen ejaculates were obtained from each boar: K – 5 units, D_1 – 5 units, D_2 – 5 units. The ejaculates were assessed to examine the following physical characteristics: ejaculate volume, spermatozoon concentration, percentage of sperm cells displaying progressive forward motility, total sperm cell count in ejaculate and insemination dose number per ejaculate. The collected ejaculates were also evaluated for pH level and alkaline phosphatase activity by Cormay Plus device using the commercial Cormay tests. The obtained research results were analyzed statistically by t-Student test.

RESULTS AND DISCUSSION

The research findings concerning the influence of vitamin C on the physical traits, pH and alkaline phosphatase activity in the ejaculates generated by duroc breed boars and duroc × hampshire and duroc × pietrain crossbreds were summarized in Tables 1 and 2. The experimental groups displayed a significant increase of semen volume obtained from all the boars. The highest ejaculate volume growth, i.e. by 53.25 ml, was observed in the duroc × hampshire crosses in the experimental group (D_1). Whereas, sperm cell concentration in 1 ml ejaculate varied between the aforementioned experimental groups, subject to a group – D_1 or D_2. The highest rise of sperm concentration in 1 ml ejaculate by 87.18 mln/ml was noted in the boars duroc × pietrain breed in the ex-

perimental group (D). Sperm cell count in total ejaculate under study was shown to increase in all the experimental boar groups (D_1 and D_2). The highest spermatozoon count growth in total ejaculate by 17.86×10^9 was noted in the boars of duroc breed from the experimental group (D1). Changes in sperm count in a single dose appeared to be highest in the boars duroc x hampshire breed in group (D_1) and statistically significant. The significant differences also concerned a number of doses generated by the boars duroc breed and duroc × hampshire crosses. The greatest statistically highly significant improvement in this range occurred in the duroc × hampshire crossbreds in the experimental group (D_1) – by 7.25 units, followed by that observed in the duroc boars in group (D_1) – by 4.66 units. While, the highest rise in a percentage of sperm cells with forward progressive motility was reported in the duroc boars in the experimental group (D_1) – by 4.44%. There was also noted elevated pH of ejaculate, whose value was presented as arithmetic mean calculated for total ejaculates from boars of a given genotype that underwent dietary vitamin C administration (Table 2). The highest pH elevation was observed in ejaculate from duroc × hampshire boar crosses – by 0.31, whereas the lowest in duroc breed – by 0.18. Alike, the lowest increase in phosphatase enzyme activity occurred in duroc x hampshire boar crosses – by 3 570 U/l and in duroc × pietrain by 4 360 U/l, while the highest by 11 760 U/l was recprded in duroc breed boars.

The present research, apart from an increase in ejaculate volume, has also displayed growth in total sperm cell count under study and their concentration as well as a rise in percentage of spermatozoa exhibiting progressive motility, elevated dose number and increase spermatozoon count per dose.

All of these results imply that value of the changes was dependent on a breed and boar crossing [Wysokińska and Kondracki 2000] and was likely to emerge from the genetic conditions [Kondracki *et al*. 2003, Kozdrowski and Dubiel 2004b]. The changes noted in the semen parameters could also be attributed to a vitamin C dose supplied to boars. An increased vitamin dose declined the obtained growth of the boar semen parameters. However, a vitamin C-induced increase in alkaline phosphatase activity implicated the growth of percentage of spermatozoa showing forward motility due to enhanced fructose production under the above mentioned enzyme; fructose is the major energy source for seminal cells. Vitamin C was shown to elevate pH level in boar semen which was associated with responsive reduction of lactic acid production [Krasińska-Czerlunczakiewicz 1995, Heugten 2004, Pion *et al*. 2004] in ejaculate.

The present researches have indicated a protective effect of vitamin C, as an antioxidative agent, that can inhibit oxidative stress [Golden *et al*. 2002] causing severe sperm cell dysfunction and thus, being one of critical contributors to male infertility [Jedlińska-Krakowska, 2005]. Lin *et al*. [1985] and Close and Cole [2001] reported that dietary vitamin C supplementation improved to some extent semen quality of boars exposed to repeated high ambient temperatures. Similarly, Ivos *et al*. [1971] explained an increase of gilt conception rate in summer by elevated supply of vitamin C at that time. Lin *et al*. [1985] associated the elevation of total sperm cell count in ejaculate with extra supply of vitamin C in summer season. Greer *et al*. [1987] conducted the studies at 5 different farms where half of gilts and boars received vitamin C (4 g/unit/day) during summer period. However, no marked improvement in gilt performance was noted. Owsianny *et al*. [2000] obtained fully consistent results with those presented above and stated that boar semen traits have no substantial influence on fertility of mated gilts.

Table 1. Effect of dietary vitamin C supplement to boars on chosen ejaculate traits
Tabela 1. Wpływ podawania knurom witaminy C na wybrane wskaźniki jakości ejakulatu

Specification Wyszczególnienie	Duroc			Duroc × Hampshire			Duroc × Pietrain		
	C	D_1	D_2	C	D_1	D_2	C	D_1	D_2
	mean SD	mean SD	mean SD	mean SD	mean SD	mean SD	mean SD	mean SD	mean SD
Volume of ejaculate (ml) Objętość ejakulatu (ml)	154.00^{A} 4.54	179.66^{B} 30.47	172.55^{B} 6.62	166.75^{A} 30.00	220.00^{B} 4.40	183.11^{B} 6.20	161.02^{Aa} 20.21	183.46^{B} 29.29	167.12^{a} 19.20
Concentration of spermatozoa (mln/ml) Koncentracja plemników (mln/ml)	350.00^{A} 7.29	408.77^{B} 6.86	407.55^{B} 31.81	436.66^{A} 7.20	523.44^{B} 6.54	517.75^{B} 30.80	489.12^{A} 23.24	576.30^{B} 33.14	543.79^{B} 24.35
Forward progressive motility (%) Ruch postępowy (%)	80.00^{a} 7.83	84.44^{b} 5.21	82.20^{a} 4.60	86.66^{a} 7.30	90.00^{b} 4.50	88.75^{a} 5.10	82.55^{a} 28.53	86.25^{a} 29.86	84.44^{a} 30.43
Sperm count in total ejaculate (mld) Liczba plemników w całym ejakulacie (mld)	43.10^{A} 7.54	60.96^{B} 3.48	59.25^{B} 8.78	73.96^{A} 3.80	80.16^{B} 7.40	79.98^{B} 8.60	67.79^{A} 6.60	74.26^{B} 5.80	73.96^{B} 5.60
Sperm count in dose of ajaculate (mld) Liczba plemników w dawce ejakulatu (mld)	3.10^{A} 0.11	3.41^{B} 0.10	3.23^{A} 1.13	3.10^{Aa} 0.10	3.92^{B} 0.11	3.60^{b} 1.12	3.69^{a} 1.24	4.0^{a} 1.18	3.75^{a} 1.15
Number of doses of ejaculate Liczba dawek ejakulatu	14.00^{A} 1.76	18.66^{B} 4.54	16.66^{B} 1.56	18.75^{A} 4.55	26.00^{B} 1.60	21.11^{B} 1.62	18.42^{a} 2.60	19.87^{b} 2.40	18.66^{a} 2.56

A, B – means denoted with capital letters differ significantly at $p \leq 0,01$
A, B – średnie oznaczone dużymi literami różnią się istotnie przy $p \leq 0,01$
a, b – means denoted with small letters differ significantly at $p \leq 0,05$
a, b – średnie oznaczone małymi literami różnią się istotnie przy $p \leq 0,05$
C – control, D1 and D2 – experimental groups
K – grupa kontrolna, D_1 i D_2 – grupy doświadczalne

The research findings, though, have revealed that vitamin C affects the L-carnitine synthesis, which in turn has beneficial impact on all the analyzed semen traits [Rebouche 1991, Kozink *et al*. 2004]. The present studies have also indicated that through the L-carnitine synthesis, vitamin C has direct and indirect influence on an increase of ejaculate volume, spermatozoon count in total ejaculate, percentage of sperm cells exhibiting forward progressive motility and elevated number of usable doses, which have been confirmed by the research results obtained by the present authors. Importantly, value of changes was dependent on a boar genotype and vitamin C dose supplied to animals. Number of semen doses obtained from a single ejaculate is related to its volume, sperm cell concentration in ejaculate and motility [Banaszewska and Kondracki 2005]. These authors report that a number of insemination doses obtained is crucial from an economic viewpoint. Therefore, a number of usable doses from a single ejaculate is important as it affects a unitary cost of a semen dose. A greater number of usable insemination doses decreases proportionally variable costs of boar management. Enzymes secreted in the

male accessory gland tissues convert glucose into fructose [Stryer *et al*. 2007]. A major role in this process is attributed to alkaline phosphatase present in seminal vesicle secretion which breaks down phosphohexoses to phosphoric acid and free fructose. The most important process that supplies spermatozoon energy proves to be the fructose breakdown – fructolysis. Alkaline phosphatase, a constituent of ejaculate, is one of the major enzymes that provides indirectly energy to sperm cells. Activity of this enzyme varies subject to, among others daily light fluctuations which was observed in boar semen by Kozdrowski and Dubiel [2004a].

Table 2. Effect of dietary vitamin C supplement to boars on pH and alkaline phosphatase activity in ejaculate
Tabela 2. Wpływ podawania witaminy C knurom na pH i aktywność fosfatazy zasadowej w ejakulacie

Boar breed Rasa knura	pH						Alkaline phosphatase activity U/l Aktywność fosfatazy zasadowej U/l					
	C		D_1		D_2		C		D_1		D_2	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Duroc	7.20^{A}	0.08	7.44^{B}	0.45	7.38^{B}	0.21	$33\ 240^{A}$	3 139	$45\ 000^{B}$	2 769	$38\ 520^{B}$	3 250
Duroc × hampshire	7.29^{A}	0.15	7.60^{B}	0.60	7.52^{B}	0.20	$42\ 780^{A}$	3 266	$50\ 460^{B}$	2 935	$46\ 350^{B}$	3 570
Duroc × pietrain	7.25^{A}	0.14	7.51^{B}	0.30	7.45^{B}	0.27	$39\ 240^{A}$	3 441	$46\ 920^{B}$	3 492	$43\ 600^{B}$	2 985

A, B – means denoted with capital letters differ significantly at $p \leq 0.01$
A, B – średnie oznaczone dużymi literami różnią się istotnie przy $p \leq 0{,}01$

Another agent increasing alkaline phosphatase activity level appears to be vitamin C whose effect was the objective of the present study. The research results illustrating its activity (Tab. 2) were consistent with those given by Turner and McDonnell [2003] and Głogowski *et al*. [2002]. An enzyme, seminal alkaline phosphatase breaks down phosphohexoses to phosphoric acid and fructose which solely in mammals, according to Stryer *et al*. [2007], is utilized as an energy source. A vitamin C dietary supplement in male boars improved a percentage of spermatozoa exhibiting forward progressive motility in all the boar genetic groups investigated (Tab. 1). Sperm cells with enzyme contribution break down fructose, glucose and mannose to lactic acid. The studies by Krasińska-Czerlunczakiewicz [1995] and Heugten [2004] and Pion *et al*. [2004] demonstrate that vitamin C promotes a decline of a blood lactic acid level. A similar process is assumed to proceed in the semen of duroc breed boars and duroc × Hampshire and duroc × pietrain crossbreds where pH level was demonstrated to grow (Tab. 2). However, elevated vitamin C dose supplied to the boars implicated some changes in the semen parameters increase obtained under the vitamin introduction. The present researches have revealed that vitamin C supplement to boar diet which apparently improves male fertility is a matter of major importance.

CONCLUSIONS

1. The study on vitamin C effect on ejaculate quality has indicated not only increased ejaculate volume but elevated total ejaculate spermatozoon count and their concentration as well as higher percentage of forward motile sperm cells, greater number of insemination doses and increased sperm cell count per dose. The changes were breed- and boar crossing-dependent.

2. Value of changes of each boar ejaculate parameter was subject to supplied vitamin C rate. Increased dietary vitamin C dose has changed the parameter values obtained on its application.

3. Elevation of alkaline phosphatase activity under vitamin C impact increased a percentage of sperm cells displaying forward progressive motility.

4. Vitamin C has also been demonstrated to rise pH level in boar semen.

REFERENCES

Banaszewska D., Kondracki S., 2005. Rozwój płciowy knurów inseminacyjnych. Prz. Hod., 10, 8–10.

Close W.H., Cole D.J.A., 2001. Don't ignore the boar! Minerals and vitamins for breeding boars. Feed Mix. 9, 1, 8–11.

Dabrowski K., Hinterleitner S., 1989. Application of a simultaneous assay of ascorbic acid, dehydroascorbic acid and ascorbic sulphate in biological materials. Analyst, 114, 1, 83–87.

Głogowski J., Danforth D.R., Ciereszko A., 2002. Inhibition of alkaline phosphatase activity of boar semen by pentoxifylline, caffeine, and theophylline. J. Andrology, 23, 6, 1–15.

Golden C., Rosenkrans C., Johnson Z., 2002. Effects of ascorbic acid and alpha-tocopherol on cryopreserved boar sperm. Arkansas Anim. Sci. Dep. Rep., 499, 120–123.

Greer E.B., Gardner I.A., Wright G.L., 1987. Failure of dietary vitamin C supplementation to prevent seasonal infertility in pigs. Aust. J. Exp. Agric., 27, 343–347.

Heugten E., 2004. Can vitamin C improve pork quality? Swine News. 27, 8, 34–38.

Ivos J., Doplihar C., Muhaxhiri G., 1971. Thermic stress as a factor of disturbances in the reproduction of pigs and possibility of prevention of these disturbances by the addition of ascorbic acid. Vet. Archiv., 41, 202–216.

Jacyno E., Kawęcka M., Kamyczek M., 2003. Wpływ selenu organicznego i witaminy E na przydatność rozpłodową młodych knurów. Trzoda Chlew., 4, 40–43.

Jedlińska-Krakowska M., 2005. Stres oksydacyjny oraz wpływ reaktywnych form tlenowych na funkcję nasienia. Med. Wet., 61(10), 1122–1123.

Kondracki S., Banaszewska D., Mielnicka C., 2005. The effect of age on morphometric sperm traits of domestic pigs (Sus scrofa domestica). Cell. Mol. Biol. Lett., 10, 3–13.

Kondracki S., Banaszewska D., Wysokińska A., Kopiś M., 2004. Wpływ wieku na właściwości nasienia knurów rasy pietrain użytkowanych w inseminacji. Zesz. Nauk. Prz. Hod., 72, 2, 69–76.

Kondracki S., Wysokińska A., Czeczot M., 2002. Ocena jakości ejakulatów knurów ras hampshire i pietrain oraz mieszańców (hampshire × pietrain) z uwzględnieniem wpływu pory roku. Ekołogo-ekonomiczni problemi rozwitu APK. Lwów, t. 2, 466–473.

Kondracki S., Wysokińska A., Kowalczyk Z., 2003. Wpływ krzyżowania ras duroc i pietrain na cechy ejakulatów knurów mieszańców dwurasowych. Zesz. Nauk. Prz. Hod., 68, 2, 105–112.

Kondracki S., Wysokińska A., Paplińska A., 2000. Wpływ wieku na cechy nasienia młodych knurów rasy wielkiej białej polskiej (Influence of the age on semen indices of young Polish Large white boars). Zesz. Nauk. PTZ, 48, 103–110.

Kozdrowski R., 2004. Wpływ pory roku na jakość nasienia knurów. Trzoda Chlew., 2, 31–33.
Kozdrowski R., Dubiel A., 2004a. The effect of season on the properties of wild boar (Sus scrofa L.) semen. Anim. Reprod. Sci., 80(3-4), 281–289.
Kozdrowski R., Dubiel A., 2004b. Właściwości nasienia mieszańców dzika ze świnią domową w cyklu rocznym. Med. Wet., 60(1), 57–61.
Kozink D.M., Estienne M.J., Harper A.F., Knight J.W., 2004. Effect of dietary L-carnitine supplementation on semen characteristics in boars. Theriogenology. 61, 7/8, 1247–1258.
Krasińska-Czerlunczakiewicz H., 1995. Wpływ dożylnego podawania kwasu askorbinowego na zachowanie się stężenia glukozy, fruktozy, kwasu mlekowego i pirogronowego we krwi pacjentów z ostrymi niedokrwiennymi schorzeniami mózgu w najwcześniejszym okresie choroby. Pr. doktorska. AM Lublin.
Lin H.K., Chen S.Y., Huang C.Y., Kuo Y.H., Wung LC., 1985. Studies on improving semen quality of working boars fed diets with addition of vitamin C in summer season. Ann. Res. Rep. Anim. Res. Inst. Taiwan Suger Corp., 73/74, 59–73.
Owsianny J., Dziadek K., Czarnecki R., Kawęcka M., Jacyno E., 2000. Wpływ libido i cech nasienia knurów linii 990 i rasy duroc na płodność krytych nimi loch. Biul. Nauk., 7, 209–216.
Pion S.J., van Heugten E., See M.T., Larick D.K., Pardue. S. 2004. Effect of vitamin C supplementation of plasma ascorbic acid and oxalate concentrations and meat quality in swine. J. Anim. Sci. 82, 2004–2012.
Rebouche C.J., 1991. Ascorbic acid and carnitine biosynthesis. Am. J. Clin. Nutr. 54, (6 Suppl.), 1147S–1152S.
Sindair S., 2000. Niepłodność u mężczyzn: rola czynników żywieniowych i środowiskowych. Altern Med. Rev., 5 (1), 28–38.
Stryer L., Tymoczko J. L., Berg J. M., 2007. Biochemia. Wyd. Nauk. PWN. Warszawa.
Trudeau V., Sanford L.M., 1986. Effect of season and social environment on testis size and semen quality of the adult Landrace boar. J. Anim. Sci., 63(4), 1211–1219.
Turner R.M.O., McDonnell S.M., 2003. Alcaline phosphatase in stallion semen: characterization and clinical applications. Theriogenology, 60, 1–10.
Więckowski W., 1980. Badania nad poziomem kwasu L-askorbinowego i jego niedosytem u świń. Rozpr. hab., Instytut Weterynarii, Puławy.
Wysokińska A., Kondracki S., 2000. Jakość nasienia knurów różnych ras (Semen quality of boars of different breeds). Naukowyj Wysnik Lwiwskoj Derżawnoj Akademii Weterinarnoj Mediciny, t. 2, cz. 3, 256–258.

Streszczenie. Knury rasy duroc i ich mieszańce duroc × hampshire oraz duroc × pietrain otrzymywały witaminę C w ilości 2,5 i 3,6 g/szt./dzień przez okres 30 dni. Przed podaniem witaminy C pobierano ejakulaty od samców, które stanowiły grupę kontrolną (K). Po podaniu knurom witaminy C ponownie pobierano ich ejakulaty, które stanowiły grupę doświadczalną (D). W eksperymencie oprócz zwiększenia objętości ejakulatu osiągnięto zwiększenie ilości plemników w całym badanym ejakulacie i ich koncentracji, również zwiększenie odsetka plemników wykazujących prawidłowy ruch, a także zwiększenie liczby dawek ejakulatu przeznaczonych do unasienniania i liczby plemników w pojedynczej dawce, przy czym wielkość tych zmian uzależniona była od genotypu knura. Wraz ze zwiększeniem dawki witaminy C podnosiła się także wartość pH w nasieniu knurów.

Słowa kluczowe: witamina C, nasienie, knury