



Effects of Selenium Injection on the Reproductive Performance of Holstein Dairy Cows

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Abstract | This study aimed to determine the effects of selenium (Se) injection on 1) the Se concentration in blood, 2) the body condition score, 3) the number of artificial fertilizations per conception, and 4) the days open of dairy cows. In the conduct of the study, forty-two (42) Holstein cows were divided into control and treatment groups. The treatment groups were classified based on the parity of the dairy cows (2~3, 4, 5, and 6~7 parity). The control group was fed with basal diet while the treatment groups were fed with basal diet plus 40 mL of Se injection. The results showed that the Se concentration in blood was greater in treatment groups than that in the control group ($p < 0.05$). The average Se concentrations in 2~7 parity treatment groups and the control group were 0.24 and 0.16 mg/L, respectively. The effects of Se injection on the number of artificial inseminations per conception and on the days open of dairy cows, on the other hand, resulted to be lower in the treated cows compared to the untreated cows or control group. The average number of artificial inseminations per conception in the 6~7 parity treatment group (1.50) lowered by half than the control group (3.67). The treated cows also showed a decrease in days open as compared to the untreated cows. The days open decreased by an average of 13.35 days in the treatment groups (126.96 days) compared to the control group (140.31 days). In conclusion, these results indicate that the injection of Se to dairy cows might affect the parameters of their reproduction such as the number of artificial inseminations per conception and days open.

Keywords | Selenium, Injection, Dairy cows, Reproductive, Parity

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INTRODUCTION

With the recent increase in national income and economic level worldwide, the consumption of meat and milk products has significantly increased. However, each country's dairy industry has been faced with international trade pressures such as the World Trade Organization and the free trade agreements. As of the fourth quarter of 2017 in Korea, the number of dairy farmers has decreased by 44 farmers (0.7%) from its 6,070 dairy

farmers on the previous quarter. On the other hand, the number of dairy heads was increased by 7,000 (1.8%) from its 417,000 dairy heads on the previous quarter. Despite the steady decline in the number of dairy farmers, more than 50 medium and large dairy farmers have increased their production. In fact, the average daily milk production in 2017 was 5,825 tons which was higher than in 2009 (2,249 tons). The yield of milk per cow is rapidly increasing due to the high milking capacity of dairy cows, but the reproductive efficiency is decreasing every year (Roche et

There are several factors that determine the economic performance of dairy cows. Some of these are the economy, the high estrus detection rates, the reasonable maintenance of first fertilization days after delivery, and the ideal fertility rates which require a 12-month delivery interval for dairy cows (Pelssier, 1976). The reproductive efficiency of dairy cows is important for income growth and sustained dairy farming. Thus, various methods for improving the reproductive efficiency of dairy cows have been studied but it was found that high milk-producing cows have had problems with low fertility and infertility (Jong et al., 1996; Whitfield, 2020). In particular, the first estrus and postpartum rates after delivery are closely related to the milk flow rate in dairy cows (Erb et al., 1980; Stokes et al., 2019). In order to prevent or treat these reproductive disorders, selenium is added to animal feed in the form of additives. Selenium (Se) is a semi-metal having a similar chemical structure as that of sulfur (S). It is a group 6b element with an atomic number of 34 and an atomic weight of 78.96u. It can also be presented as either selenite (Se⁴⁺) or selenate (Se⁶⁺) in its reduced form.

In dairy cows, selenium deficiency causes a number of disorders. The most well-known of which are myopathy, stillbirth, postpartum congestion (Kamada, 2017), and low pregnancy rates (Larson et al., 1980; Juniper et al., 2019). There are reported reproductive factors affected by selenium deficiency in the blood. These factors include decreased levels of progesterone (Buck et al, 1981; Kamal et al., 2020), decrease in conception rate (Buck et al, 1981; Khalili et al., 2019), and increase in day open period (Ropstad et al., 1988; Khatti et al., 2017). These show that reproductive decline and increased disease resulting from selenium deficiency in dairy cows can be a significant problem.

The utilization of selenium in feed is much lower in ruminants than in other animals (Kim, 2000; Kim and Mahan, 2001). When introduced in the body, absorbed selenium is transported and accumulated through the blood to various tissues in the body, with the highest concentrations in the liver and kidneys (Ullah et al., 2020). The selenium concentration in the blood of an animal indicates the short time nutritional status of selenium while the selenium concentration in the tissue can represent its long-term nutrition status. Ruminants have different excretion pathways for selenium depending on how it is fed. When selenium is ingested through feed, most of it is excreted through feces, but selenium administered by injection is mainly excreted through urine (Galbraiyh et al., 2015; Combe, 2015).

Many studies have been conducted on the relationship between selenium and reproduction of animals. However, little research has been conducted on the effects of selenium

injections on factors related to reproduction of dairy cows. Therefore, this study sought to investigate on the effects of selenium injection on the reproduction of dairy cows and to use the results to establish a theoretical basis to improve farm income.

MATERIALS AND METHODS

ETHICAL PERMIT

This study was carried out in strict accordance with the recommendation of the guide for the animal experiment and management from the Animal and Plant Quarantine Agency. The protocol was approved by the Committee on the Ethics of Animal Experiments of Hankyong National University (Permit Number: HKNU-2020-0003).

ANIMALS AND FEED

Forty-two Holstein cows (650 ± 20kg) were used for this study. There were 21 heads selected for each selenium treated group and selenium untreated group. They were classified based on their parity differences (1~7). Each animal was fed with a total of 12 kg dry matter (DM)/day of concentrate on which 7 kg was quantitatively fed every 24 hours by an automatic feeding system and the other 5 kg was fed by adding a total mixed ration (TMR). Another 24 kg DM/day of roughage was fed per head by TMR. The chemical composition of the experimental feeds is shown in Table 1 and was analyzed by the AOAC method (AOAC International, 2005). Water and mineral blocks, on the other hand, were also provided and were accessed freely. The body condition score (BCS) of the selenium treated and an untreated group was measured three times (beginning, middle (150 days after selenium injection) and end of experiment).

SELENIUM INJECTION AND ANALYSIS

The dairy cows used in this study were injected intramuscularly with 40 mg selenium on to their hips (*Gluteus maximus*) with a two week interval. After which, blood samples were collected 24h post-injection for the analysis of their blood selenium concentration. Blood sampling of the dairy cows were collected between 2-4 p.m. and were taken in triplicate from the jugular vein. Each blood sample was contained in a heparin-treated 15 mL vacutainer tube and were stored in the freezer at -20 °C. For the analysis, atomic absorption spectrophotometer was used to measure the selenium concentration in blood at 196 nm (Perkin-Elmer, USA Analyst 800). Samples were diluted 20-fold with 0.05% Triton X-100 (Sigma-Aldrich, USA) and the selenium concentrations were measured using the standard method. Treatment and analysis of the selenium content in blood were performed based on the method of Zahrazadeh et al. (2018).

Table 1: Ingredients and chemical compositions of experimental diets for Holstein lactating cows (*in vivo* %, DM basis)

Chemical composition (%)	Ingredients							
	Concentrate mix ¹	TMR						
		Protein hull	Cotton seed	Oat	Clinehay	Timothy hay	Beet pulp	Mineral mix ²
Dry matter	89.24	89.24	90.72	89.96	92.35	92.36	87.40	Free choice
Crude protein	20.0	19.02	20.36	5.43	8.59	9.25	10.28	
Crude fat	4.0	2.04	17.32	1.98	1.53	2.05	0.67	
Crude fiber	12.0	10.88	25.01	26.38	27.99	30.24	20.06	
Crude ash	10.0	4.76	3.58	3.76	7.67	6.48	3.77	
NDF	29.0	13.00	47.04	32.79	33.16	35.90	26.91	
ADF	14.0	40.14	58.75	57.02	67.03	65.34	41.51	

¹ Commercial concentrate which was manufactured for lactating cows producing 30-40 kg milk per day.

² Contains 200 mg manganese, 100 mg cobalt, 4,000 mg sulfur, 150 mg iodine, 2,000 mg iron, 100 mg zinc, 100 mg copper, 50 mg nickel, 2,000 mg calcium, 3,000 mg magnesium, and 40 µg selenium.

REPRODUCTIVE PERFORMANCES AND ARTIFICIAL INSEMINATION ANALYSES

The dairy cows were classified into selenium treated and untreated groups. Each group were examined based on the following parameters; the number of days open after delivery, the parity number, the first fertilization day after delivery, the fertilization number of each pregnancy, and the BCS (Zahrazadeh et al., 2018). All animals were impregnated using artificial insemination (AI). When the experimental animal did not return to estrus within 60 days after AI, pregnancy was evaluated by rectal examination (Vedovatto et al., 2019).

Moreover, in order to increase the objectivity of this study, external environmental factors were eliminated. The body condition of each animal was considered and the seasonal environmental factors of the selenium treated and untreated groups were tested under the same conditions.

STATISTICAL ANALYSIS

Statistical analysis was carried out using the Statistical Analysis System (SAS, 2000). T-test was used to determine significant differences between the mean values of the treatment (selenium treated) and the control (selenium untreated) groups. Statistical significance was set at $p < 0.05$.

RESULTS

EFFECT OF SELENIUM INJECTION ON SELENIUM CONCENTRATION IN DAIRY COWS

Table 2 shows the effect of selenium injection on selenium concentration by parity in dairy cows. Selenium concentrations in the 2nd and 3rd parity of dairy cows were significantly higher in the treatment group (0.24 mg/L) than in the control group (0.18 mg/L; $p < 0.05$).

Even in the 4th and 5th parity of dairy cows, selenium con-

centrations recorded were 0.25 and 0.23 mg/L, respectively, for the treatment groups which are significantly higher than in the selenium concentrations recorded for the control group with 0.17 and 0.19 mg/L, ($p < 0.05$), respectively. The comparison of the control and treatment groups for the total mean blood selenium concentrations from the 2nd to 7th parities was significantly lower in the control group (0.17 mg/L) than in the treatment group (0.24 mg/L), ($p < 0.05$).

Table 2: Effects of selenium injection on selenium concentrations in blood of dairy cows

Parity number ⁴	Selenium concentration (mg/L)		SEM ³	P-values
	Control ¹	Treatment ²		
2~3	0.18	0.24*	2.95	0.002
4	0.17	0.25*	4.17	< 0.001
5	0.19	0.23*	1.67	0.040
6~7	0.16	0.24*	4.00	0.019
Average	0.18	0.24*	3.19	< 0.001

Significant difference between control and treatment ($p < 0.05$)

¹ Without injection of selenium

² Injection of selenium (40 mL per cow)

³ Pooled standard error of mean

⁴ n = 42 (Control: 21 dairy cows, Treatment: 21 dairy cows)

* indicate a significant difference as compared to control.

EFFECT OF SELENIUM INJECTION ON BCS IN DAIRY COWS

The effect of selenium injection on BCS in dairy cows is shown in Table 3. In the case of the 2nd and 3rd parities, BCS was higher in the control group (3.33) than in the treatment group (3.25), but statistically has no significant difference ($p > 0.05$). In the 4th parity, BCS tended to be higher in the treatment group (3.38) than in the control group (3.18), ($p > 0.05$). In the 5th parity, BCS was 3.05 in the control group and 3.03 in the treatment group. The

mean BCS in the 6th ~ 7th parities was 2.87 in the control group and 3.00 in the treatment group, which was not significantly higher ($p > 0.05$). The mean BCS of the 2nd ~ 7th parities was 3.17 in the control group and 3.11 in the treatment group, which was not significantly higher in the control group ($p > 0.05$).

Table 3: Effects of selenium injection on BCS of dairy cows

Parity number ⁴	BCS		SEM ³	P-values
	Control ¹	Treatment ²		
2~3	3.33	3.25	0.42	0.512
4	3.18	3.38	0.10	0.344
5	3.05	3.03	0.01	0.890
6~7	2.87	3.00	0.07	0.556
Average	3.11	3.17	0.03	0.681

¹ Without injection of selenium

² Injection of selenium (40 mL per cow)

³ Pooled standard error of mean

⁴ n = 42 (Control: 21 dairy cows, Treatment: 21 dairy cows)

EFFECT OF SELENIUM INJECTION ON THE NUMBER OF AIs PER CONCEPTION IN DAIRY COWS

The effect of selenium injection on the number of AIs per conception in dairy cows is shown in Table 4. The average number of AIs completed per conception in the treated group (avg: 1.80) was lower than that of the control group (avg: 2.44), which was found to be not significant ($p > 0.05$). The control group of the 2nd to 3rd parity cows was fertilized by AI 3.14 times, but the treated group (2.50) showed a fertilization rate 0.64 times faster than that of the control group. This indicates a higher tendency of fertilization for the treated group. In the 4th parity, an average of 2.00 AIs were required for fertilization in the control group. However, the treatment group showed a tendency to be low, with an average of 1.80. In particular, fertilization of 6th and 7th parity cows in the treatment group required only 1.50 AIs for fertilization, which was 2.17 times lower than that of the control group (3.67).

Table 4: Effects of selenium injection on the number of AIs per conception of dairy cows

Parity number ⁴	Number of AIs per conception		SEM ³	P-values
	Control ¹	Treatment ²		
2~3	3.14	2.50	0.32	0.538
4	2.00	1.80	0.10	0.798
5	1.50	1.40	0.050	0.853
6~7	3.67	1.50	1.08	0.340
Average	2.44	1.80	0.32	0.231

¹ Without injection of selenium

² Injection of selenium (40 mL per cow)

³ Pooled standard error of mean

⁴ n = 42 (Control: 21 dairy cows, Treatment: 21 dairy cows)

EFFECT OF SELENIUM INJECTION ON DAYS OPEN IN DAIRY COWS

Table 5 shows the effect of selenium injection on days open in dairy cows. Days open is related to productivity improvement of dairy farms. This is the reason why most dairy farmers are trying to minimize the days open. When selenium was injected to the 2nd and 3rd parity dairy cows, the average days open was 147 days. However, cows without selenium showed an average of 162.33 days. This shows that the days open period of cows treated with selenium was reduced by 15.33 days compared to untreated cows ($p > 0.05$). In the 4th parity, the days open of the treatment group was 89 days, which was 33 days less than that of the control group with 122 days, but not significantly less ($p > 0.05$). The injection of selenium caused around 10 days shorter days open period in the treatment group compared to the control group.

Based on the results of this study, the selenium concentration in blood was significantly increased due to the injection of selenium ($p > 0.05$). On the other hand, the average days open and the number of AIs per conception has decreased without significance ($p > 0.05$).

Table 5: Effects of selenium injection on days open period of dairy cows

Parity number ⁴	Days open period		SEM ³	P-values
	Control ¹	Treatment ²		
2~3	162.33	147.00	7.67	0.599
4	122.00	89.00	16.50	0.456
5	101.25	106.80	2.78	0.858
6~7	174.67	165.05	4.83	0.886
Average	140.31	126.96	6.55	0.613

¹ Without injection of selenium

² Injection of selenium (40 mL per cow)

³ Pooled standard error of mean

⁴ n = 42 (Control: 21 dairy cows, Treatment: 21 dairy cows)

DISCUSSION

Buck et al. (1981) reported that when selenium is absorbed into the body, it accumulates in the reproductive and endocrine organs such as the ovaries, pituitary gland, and adrenal glands. Sgoifo Rossi et al. (2017) suggested that selenium and vitamin E act as antioxidants to prevent cell aging and increase tissue vitality. McGuffey (2017) also found that selenium increases the contractile force and the number of contractions in the uterus. In general, dairy cows have increased selenium levels in blood when they are infected with mastitis and when there is a high intake

of dry matter. Additionally, overly high selenium concentrations in blood have been reported to present a risk of ovarian cysts (Mohammed et al., 1991). On the other hand, the concentration of selenium in plasma is lowered after delivery, and the lack of selenium lowers the AI rate (Jukola et al., 1996; Mujawar et al., 2019). Furthermore, it has been reported that administration of selenium in dairy cows increases immunity and resistance to disease (Hurley and Doanem 1989; Zarczynska et al., 2019).

Selenium concentrations in the blood of dairy cows are generally reported to vary from 0.09 to 0.30 mg/L. If the selenium concentration is too low, the reproduction rate decreases, and when selenium concentration is too high, dairy cows show increased risk of ovarian cysts or mastitis (Jukola et al., 1996). Therefore, selenium concentrations of 0.23.0 mg/L to 25.0 mg/L may be appropriate to maintain the ability of dairy cows to reproduce smoothly.

Body condition score (BCS) is widely used as a method for evaluating the nutritional status of dairy cows. A low BCS affects reproductive performance (Markusfeld et al., 1997) by causing delays in ovarian activity (DeVries et al., 1998; Nishimura et al., 2018) and first ovulation (Beam and Butler, 1999; Hassan et al., 2017). Therefore, BCS can be considered as an important factor in cow breeding. Improved estrus detection rates, maintenance of first fertilization days after delivery, and high conception rates are required in order to increase reproductive efficiency (Wiltbank et al., 2006; Sarah et al., 2019). These problems can be identified through BCS. Generally, when BCS falls below 2.0, estrus induction and expression rate as well as reproductive efficiency are low (Lowman, 1985; Stadnik et al., 2017). Therefore, in order to improve reproductive efficiency, specific feeding management is needed to prevent the BCS level from decreasing. In this study, the average BCS of the control and treatment groups was 3.11 and 3.17, respectively, which did not affect the reproduction rate.

According to Ryu (2010), the fertility rate of dairy cows in 2005 was 50.2% and 33.8% in fresh eggs and in frozen eggs respectively. In the year 2007, the fertility rate of dairy cows was 44.2 % and 44.3% in fresh eggs and in frozen eggs respectively. This seems to be maintained at an average rate of 30-50%, which is similar to the average number of AIs per conception which ranges from 2.1 to 2.2 (Lee et al., 2000).

In this study, AI was performed using frozen eggs, and the average number of fertilizations per conception of the control group was 2.44. Conversely, the treatment group showed an average of 1.80 AIs per conception, which was lower than the average in Korea. Therefore, further studies on appropriate selenium injection levels could significantly

reduce the number of AIs in dairy cows. These results are expected to contribute to the improvement of the reproductive capacity of dairy cows.

In the United States, Holstein dairy cows have an average days open period of 135 days (Toledo-Alvarado et al., 2017). However, according to a report by Chung et al. (1991), the days open period of Korean dairy cows was generally 145 to 175 days, which has a maximum of 47 days longer than in the United States. In addition, according to a report by Lee et al. (2000), the days open period of Korean dairy cows ranged from 130.6 to 154.2 days. Taken together, the days open period of dairy cows raised in Korea is considerably longer than that of dairy cows in the United States. In this study, the average days open period of untreated dairy cows was 140.37 days, which is consistent with the above results on Korean dairy cows. Meanwhile, dairy cows treated with selenium had a shorter average days open period of 126.96 days, which was similar to that of dairy cows in the United States. Therefore, the results of this study suggest that injection of selenium after childbirth stimulates the growth of ovulatory follicles, increasing hormone balance and uterine stability, thus the days open period becomes decrease.

CONCLUSIONS

This study suggests that the number of AIs per conception and the days open period may be reduced when selenium is injected to dairy cows after delivery. This may have a positive effect on the reproductive performance of dairy cows. However, it is recommended that future study on the effect of different selenium concentration on reproductive performance of dairy cows be conducted.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

AUTHORS CONTRIBUTION

Gil Woo Han: Investigation, Experiment, Data analysis, Formal analysis. Jong Ho Ahn: Experiment, Statistical analysis, Methodology, Writing of Original draft, Review, Editing. In Sik Nam: Supervision.

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