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Original research

Adaptation of Brown Swiss cows breed in first lactation cycle

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Abstract. The intensive technology of exploitation on industrial farming places high demands on the adaptive properties of animals. Animal farming intensification requires the technologies which can provide high reproductive qualities and the realization of a genetically determined level of milk productivity combined with minimal expenditure for recovery and animal keeping. This especially applies to young cow farming where several factors are important including the adaptation to the housing conditions, the feeding quality and milking regime. At the same time, first-heifers have a high physiological load on the synthesis and secretion of milk, during which the organism continues to grow. The study of adaptive properties of Swiss breed cows in the first lactation was carried out in the conditions of the Yekaterinoslavsky dairy production complex of the Dnipropetrovsk region (Ukraine). The received data have shown that in the first two months of lactation (60.2 ± 2.18 days) Swiss first-heifers produced an average of 1680.2 kg of milk. This indicator is quite high for first-heifers, but the coefficient of variation at the level of 39.5 % indicates individual and rather different realization of the genetic potential of milk productivity. During the following two months of lactation, the milk yield of Swiss first-heifers practically doubled (P<0.001). At the same time, the individual reaction of the intensity of lactation function of cows was also high, although the coefficient of variation decreased to 14.4 %. After 4 months after calving, during the following two months of lactation, the level of milk productivity of the Swiss first-heifers increased by 1.47 times and reached the level of 4982.2 kg, which exceeded the four-month milk yield by 32.5 % (P<0.001). During an almost standard lactation (305.0 ± 2.20 days), Swiss first-heifers realized their own milk productivity at the level of 7420.5 kg. That is, during the last two-month period, the percentage of milk yield of first-heifers increased by 13.9 % (P<0.001). If the milk yield of first-heifers increased dynamically during standard lactation, its qualitative indicators, such as milk fat and milk protein content, were quite stable. The mass fraction of fat practically corresponded to breed characteristics and was at the level of 3.82–3.86 %, and protein – 3.30–3.38 %. The ratio of fat to milk solids corresponded to the norm and averaged 1.13–1.17 units. Obtained data demonstrated significant adaptability of Brown Swiss cows in respect with milk productivity potential. However, Swiss first-heifers developed the minor negative value of adaptation index.

Keywords: cows; productivity; fat and milk protein; adaptation index.

Адаптація корів бурої швіцької породи у період першої лактації

Анотація. Інтенсивна технологія експлуатації на промислових комплексах ставить високі вимоги до адаптаційних властивостей тварин, які за мінімальних можливостей відновлення і відпочинку можуть забезпечувати як високі відтворні якості, так і реалізацію генетично обумовленого рівня молочної продуктивності. Особливо це стосується молодих корів, яким необхідно адаптуватися до умов утримання, рівня та якості годівлі, а також видоювання у доїльній залі. При цьому, у самих первісток перше високе фізіологічне навантаження на синтез та секрецію молока, упродовж якого організм продовжує подальший ріст. Дослідження адаптаційних властивостей швіцьких корів у першу лактацію на умови експлуатації проведені в умовах виробничо-молочного комплексу «Єкатеринославський» Дніпропетровської області (Україна). Отримані дані показали, що у перші два місяці лактації (60,2 ± 2,18 діб) швіцькі первістки продукували в середньому 1680,2 кг молока. Цей показник як для первісток достатньо високий, але коефіцієнт варіації на рівні 39,5 % вказує на індивідуальну і досить різну реалізацію генетичного потенціалу молочної продуктивності. упродовж послідуючих двох місяців лактації удій швіцьких первісток практично подвоївся (P<0,001). При цьому, індивідуальна реакція інтенсивності лактаційної функції корів теж була високою, хоча коефіцієнт варіації знизився до показника 14,4 %. Після 4-ох місяців після отелення, упродовж послідуючих двох місяців лактації рівень молочної продуктивності швіцьких первісток практично зріс у 1,47 раза і сягнув рівня 4982,2 кг, що перевищувало показник чотирьох-місячного удою на 32,5 % (P<0,001). За практично стандартну лактацію (305,0 ± 2,20 доби) швіцькі первістки реалізували власну молочну продуктивнысть на рівні 7420,5 кг. Тобто, упродовж останнього двохмісячного періоду удій у первісток зріс на 13,9 % (P<0,001). Якщо удій молока у первісток упродовж стандартної лактації динамічно зростав, то його якісні показники, такі як жиро- і білковомолочність були досить стабільними. Масова частка жиру практично відповідала породним особливостям і знаходилася на рівні 3,82–3,86 %, а білка – 3,30–3,38 %. Співвідношення жиру до бліка молока відповідало нормі і становило у середньому 1,13–1,17 одиниці. Отримані дані показали значну пристосованість корів бурої породи за потенціалом молочної продуктивності. Проте у швіцьких первісток проявилося незначне негативне значення індексу адаптації.

Ключові слова: корови; продуктивність; жир і молочний білок; індекс адаптації.

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Introduction

The production of milk from cows is one of the main branches of agricultural production in accordance with the needs of the market. This is confirmed by the fact that the world production of cow's milk, as noted by researchers (Bauman et al., 2006), has been steadily growing for many decades both in America and in Europe. Gross (2023) researched that in the United States the productivity of cows has doubled in the last 40 years alone, which is six times more than 100 years ago. However, increasing the productivity of cows provides not only an increase in the profitability of milk production (VandeHaar, 1998; Capper at al., 2009; Gorlov at al., 2023), but is also accompanied by an increase in various diseases of dairy animals (Heuer at al., 1999; Fleischer at al., 2001; Overton at al., 2017) and, most importantly, a decrease in their reproductive function (Lucy, 2001). The fact is that the high reproductive productivity of dairy herds is rightly recognized as the main factor in the profitability of cow breeding (Oltenacu et al., 1981; Britt, 1985; De Vries, 2004; Meadows et al., 2005).

An important factor is the fact that in highly productive herds there is a reduction in the duration of economic use of dairy cows (Gröhn at al., 1998; Dallago at al., 2021; Hu at al., 2021; Warner at al., 2022). According to researchers (Coffey et al., 2004), this is especially evident in animals that are purposefully selected for high milk productivity. Pinedo et al. (2010), Carvalho et al. (2019) indicate that post-calving disease in cows significantly determines the risks of removal from the herd. De Vries et al. (2010) conclude that the low reproductive capacity of cows is defined as the main risk of their removal from the herd. Reproduction problems, as indicated by (Fleischer et al., 2001; Ingvartsen et al., 2003), create performance diseases such as hypocalcemia, ketosis, colostrum displacement, mastitis, etc. Even parturition in dairy cows can be accompanied by acute systemic inflammation and stress due to damage to uterine tissues (Trevisi et al., 2012). High loads on the orgaism of cows in conditions of intensive technology of their exploitation leads to the fact that animals are used for less than three lactations. Researchers (Hare et al., 2006) note that most animals are culled in the herd up to 6 years age.

Baumgard et al. (2017) note that the beginning of lactation in cows is the most critical phase of nutrient regulation, as many physiological adaptations occur that ensure the regulation and maintenance of high udder activity for milk synthesis and secretion. Bionaz et al. (2007), Bradford et al. (2015), Trevisi and Minuti (2018) indicate that after calving, animals need a certain period of adaptation to the transition period, which is associated with a significant increase in feed energy consumption. After calving, animals' feeding behavior changes. As noted by Neave et al. (2017), during the transition period, cows spend more time consuming feed.

However, some cows cannot cope with such an increase in energy consumption, leading to an energy imbalance, after which organism reserves are mobilized (Drackley, 1999; Cattaneo et al., 2023). Esposito et al. (2014) note that in lactating cows during the transition period there is a negative energy balance and a decrease in immunity, which can coincide, making the animals susceptible to various diseases and metabolic disorders. Researchers McArt et al. (2012) note that hyperketonemia is observed in most high-yielding dairy cows at the beginning of lactation. Moreover, animals may experience metabolic stress at the beginning of lactation (Gross et al., 2016). Even excessive lipolysis and ketogenesis in the early stages of lactation in cows, combined with a reduced immune system, increase the level of health disorders and the occurrence of various diseases (Trevisi et al., 2012). It is no coincidence that many researchers (Schultz et al., 2016; Bisinotto et al., 2016; Carvalho et al., 2019) believe that both the reproductive function and the level of milk productivity. It is natural that among animals there is a significant variability of physiological adaptation even under conditions of the same feeding and maintenance. Moreover, researchers Fleischer et al. (2001) and Ingvartsen et al. (2003) did not find a negative relationship between high productivity of animals and the occurrence of diseases in them and subsequent removal from the herd.

Thus, the optimal breeding program for repair young animals, which determines its future level of milk productivity (Soberon et al., 2012; Korst et al., 2017), metabolic load after calving, as well as heat stress (Wheelock et al., 2010), etc., is essential affect the occurrence of operational diseases and decrease in both productive and reproductive qualities of cows.

The purpose of study was the finding out of the peculiarities of Brown Swiss cow adaptation on the farms of Ukraine.

Materials and methods

The study of the adaptation properties of Swiss breed cows in the first lactation to conditions of exploitation was carried out on “Yekaterinoslavsky” farm and dairy complex of the Dnipropetrovsk region (Ukraine). At a large industrial enterprise for the production of milk, animals are kept in easily collapsible cowshed, in which full-ration fodder mixture is distributed to feed tables twice a day and milking is carried out three times a day in a milking hall with a "Parallel" type installation.

The value of a single cow milking was recorded monthly on average for a week, after which productivity was determined for a certain period or the entire lactation. Selection of the average milk sample carried out in automatic mode at the milking installation during the milking of cows. The mass fraction of fat (%) was determined for 2–3 months of lactation of cows on automatic analyzers "AKM-98" and "Ekomilk 120 – KAM 98-2A" with control by Gerber's acid method. Milk protein (%) is determined by the refractometric method on the "IRF- 454 B2M" device.

The service period (SP) of Swiss first-heifers was defined as the period from calving to effective artificial insemination (days); intercalving period, as the period from one calving to the next (days); coefficient of reproductive capacity (CRC) according to the formula: CRC = 365/IP, where IP is the average period between calvings, days. The optimal value of CRC is from 1 to 0.95.

Respectively to decision of the responsible manager of the farm corporation there was considered that the average value of the service period (SP) of cows at the level of 85 days is optimal. Every day above this value are considered as infertility. Therefore, the period of infertility (I) was determined (days): I = SP – 85.

To determine the loss of offspring due to infertility, the coefficient of how many offspring are produced per day of the animal's organism, provided that 0.0035 heads of offspring occur per day of the animal's organism (1 : 285 = 0.0035 head). Thus, offspring losses (OL) can be calculated as equation OL = I × 0.0035.

The coefficient of milk yield of animals was determined, which reflects the cow’s milk volume produced during the lactation period relatively to 100 kg of live weight. The reaction norm of cows in the interaction "genotype - environment" was evaluated according to the adaptation index: IA = (365–IP)/MF×27.40; where: IA is the adaptation index; IP – intercalving period (days); 365 – the number of days in a year; MF – milk productivity of cows for finished, shortened, or for 305 days of lactation, expressed in kilograms of milk fat; 27.40 is the coefficient.

The authors reported that the maximum value of the adaptation index can be +37.0, and the minimum – -192.0. Ideally (IP=365 days) the index is zero (Zasukha et al, 1999; Siratskyi et al, 2005). A negative value of the adaptation index indicates a violation of the balance between the environment and the animal's organism. At the end of the study, all first-heifers of Swiss cows (n=85) were divided into two groups depending on the duration of the lactation period: Group I (n = 58) – 357.7 days of lactation; II group (n = 27) – 500.1 days of lactation.

Table 1 – Dynamics of productive indicators of cows duirng periods of first lactation, n = 85 (M ± m)

Days of lactation	Milk yield per period			Mass fraction		
	kg	σ	Cv, %	milk fat, %	milk protein, %	milk fat/milk protein
60.2 ± 2.18	1680.2 ± 71.93	663.1	39.5	3.86 ± 0.032	3.30 ± 0.010	1.17 ± 0.010
120.1 ± 1.45	3362.5 ± 52.51	484.1	14.4	3.85 ± 0.037	3.37 ± 0.010	1.14 ± 0.011
180.4 ± 2.03	4982.2 ± 61.97	571.3	11.5	3.83 ± 0.040	3.34 ± 0.013	1.15 ± 0.013
240.6 ± 1.52	6383.5 ± 105.20	969.8	15.2	3.82 ± 0.041	3.38 ± 0.014	1.13 ± 0.013
305.0 ± 2.20	7420.5 ± 85.38	787.2	10.6	3.85 ± 0.050	3.35 ± 0.017	1.15 ± 0.015

For the selection of methods of biometric calculation of the results of scientific data, we focused on the purpose and tasks that were set before the beginning of the research. The obtained data were processed with using the standard package of statistical programs "Microsoft Office Excel".

Results

The main index of the cow adaptation to intensive exploitation technology in the conditions of a large industrial complex for a concentration of 1.200 lactating cows is their productive qualities, that is, the dynamics of milk production after calving during lactation. If the dynamics are positive, then we can talk about the good adaptive capacity of animals that are in satisfactory conditions of mainterance and with a high level of feeding. The given data in Table 1 show that in the first two months of lactation (60.2 ± 2.18 days) Swiss first-heifers produced an average of 1680.2 kg of milk. This indicator is quite high for first-heifers, but the coefficient of variation at the level of 39.5 % indicates individual and rather different realization of the genetic potential of milk productivity in the first months of lactation.

Despite the individual reaction of the Brown Swiss cows to the first lactation, their milk yield increased significantly in the following two months, and for a four-month period (120.1 ± 1.45 days) the average level of productivity was at the level of 3362.5 kg. That is, during the following two months of lactation, the milk yield of Swiss first-heifers practically doubled ($P<0.001$). At the same time, the individual reaction of the intensity of lactation function of cows was also high, although the coefficient of variation decreased to 14.4 %.

During the next 120–180 days of lactation, the level of milk productivity of Swiss first-heifers increased by 1.47 times and reached the level of 4982.2 kg, which exceeded the indicator of four months of milking by 32.5% ($p<0.001$).

In this period of lactation, the individual characteristics of lactating animals in the realization of milk productivity leveled off, since the coefficient of variation of milk yield in comparison with the previous indicator, although it remained at a high level, nevertheless decreased and amounted to an average of 11.5 %.

Over the course from 240 days, Swiss first-heifers secreted an average of 6383.5 kg of milk, which exceeded the rate of six monthly lactations by 21.9 % ($p<0.001$). At the same time, the coefficient of variation in the milk yield of first-heifers did not decrease as predicted, but on the contrary, it even increased to an indicator of 15.2 %. That is, in the period from 180 to 240 days of lactation, the individual reaction in realizing the level of milk yield of lactating cows to the conditions of feeding, milking and maintenance increased.

During an almost standard lactation (305.0 ± 2.20 days), Swiss first-heifers realized their own milk productivity potential at the level of 7420.5 kg. That is, during the last two-month period, the percentage of first-heifers increased by 13.9 % ($p<0.001$). The coefficient of variation in animal milk production during this period significantly decreased and did not exceed 10.6 %.

If the milk yield of the first-heifers increased dynamically during standard lactation, its qualitative indicators, such as milk fat and protein content, were stable. Thus, the mass fraction of fat practically corresponded to breed characteristics and was at the level of 3.82–3.86 %, and protein – 3.30–3.38 %.

A good indicator that characterizes the correspondence of the level and quality of feeding lactating first-heifers is the ratio of the protein to fat indicator. During the 305-day lactation period of Swiss first-heifers, this indicator corresponded to the norm and amounted to an average of 1.13–1.17 units.

Depending on the duration of completed lactation, the array of Swiss first-heifers was divided into two groups: Group I – duration of complete lactation of first-heifers at the level of 357.7 days (n = 58) and Group II – 500.1 and n = 27, respectively (Table 2). The duration of cow lactation cycle was determined by the service period and the duration of pregnancy during milking. The duration of the first lactation period was at the level of 357.7 days in the majority of first-heifers (Group I, 58 heads), as the service period was 117.7 days on average. In animals of the II group, the service period after calving lasted an average of 260 days. That is why the first lactation of these animals lasted 500.1 days. These indicators were higher in the first group by 28.5 and 54.7 %, respectively ($p<0.001$).

According to the accepted technology at the dairy complex, it is considered the norm that the cow enters the state of estrus after calving for 85 days. If this does not happen, then a program of hormonal stimulation of ovulation on the ovaries is applied to such animals. That is, management can deliberately contribute to the extension of the service period of high-performance animals. Taking into account these factors, the infertile period was still

Table 2 – Reproductive indicators of Brown Swiss cows at different durations of the lactation period

Group of animals	Lactation, days	Duration of the period			Reproductive capacity coefficient	Loss of offspring, individuals per group
		from calving to insemination	infertility	from first to second calving		
I, n = 58	357.7 ± 1.57	117.7 ± 1.57	32.7 ± 1.57	405.7 ± 1.76	0.9 ± 0.004	0.11 ± 0.006
II, n = 27	500.1 ± 14.07	260.0 ± 14.17	175.0 ± 14.57	554.0 ± 14.48	0.7 ± 0.020	0.61 ± 0.050

Table 3 – Productive qualities of Brown Swiss cows for a lactation

Group	Milk yield per period, kg	Product, kg			Milk index	Adaptation index
		milk fat	milk protein	milk fat+ milk protein		
I, n = 58	8423.4 ± 81.15	331.0 ± 5.52	282.8 ± 2.90	593.8 ± 7.98	2124.0 ± 28.57	-3.7 ± 0.24
II, n = 27	10132.6 ± 308.45*	416.1 ± 18.85	347.7 ± 10.71	763.7 ± 28.21	2575.8 ± 96.91	-11.8 ± 0.84

Note: 1. * – $p<0.001$ in respect with to the I group

determined in cows. In the first-heifers of the I group, this period lasted an average of 32.7 days, while in the animals of the II group it was 5.4 times longer ($p<0.001$) and amounted to an average of 175 days. At the same time, if in the animals of the I group the loss of offspring amounted to 0.11 heads, then in the cows of the II group they already amounted to 0.61 heads.

It was natural that the reproductive capacity of first-heifers differed for different durations of the intercalving period. If cows of the II group had an average reproductive capacity of 0.7 units, then in the animals of the I groups it was higher by 22.2 % and amounted to 0.9 units.

The reproductive function of Brown Swiss firts-heifers after the first calving practically determined their indicators of productive qualities (Table 3). Thus, during the first completed lactation, Swiss cows of the I group secreted an average of 8423.4 kg. At the same time, animals of the II group had this indicator higher by 16.9 % ($p<0.001$), which was at the level of 10132.6 kg.

Different indicators of milk yield per lactation with practically equal values of the mass fraction of fat and protein in milk determined different values of their production in animals of the two groups. Thus, if the first-heifers of the I group produced an average of 593.8 kg of milk fat and protein, it was 22.3 % higher in the animals of the II group ($p<0.001$). If the milk yield index was 2124.0 kg on average in animals of the I group, it was higher by 17.5 % ($p<0.001$) and amounted to 2575.8 kg in the cows of the II group.

The value of the reaction norm of Brown Swiss cows in the interaction "genotype – environment" in the first lactation was at a relatively normal level. Thus, if the adaptation index was -3.7 units in the cows of the I group, then in the cows of the II group it was 3.2 times higher and amounted to -11.8 units on average, but it was also within the normal range.

Discussion

Progress in the dairy industry at the end of the twentieth century was focused on a significant increase in milk production based on the latest technological developments in the milking system of cows with the possibility of increasing its frequency (de Koning et al., 2002). And this means that the first-heifers, after transferring the natural load during calving, face a new load, which is connected with the removal of milk from their udders by the milking machine. Some researchers indicate (Rushen et al., 2001) that the mechanization of the milking process can lead to an increase in the level of cortisol in the blood plasma, an increase in heart rate, a decrease in the release of oxytocin and, as a result, a decrease in milk yield and an increase in the proportion of residual milk in the udder of cows. At the same time, Tancin et al. (2001) note that the reaction to milking largely depends on the individual reaction of a single animal. In cows at the very beginning of lactation, the milk yield reflex may be inhibited due to stress caused by milking in an unfamiliar environment (Rushen et al., 2001). Researchers Breuer et al. (2000) hypothesize that there is a negative relationship between cow fear and performance. That is, individual variations in the behavioral and physiological reactions of dairy cows to milking are significant. Nevertheless, the conducted studies showed that after calving, the level of milk productivity of Swiss first-heifers did not indicate inhibitory processes of synthesis and secretion of milk in the udder, since during the first two months of lactation, the average daily milk yield was at a high level and did not fall below the indicator of 27.9 kg .

The next two months of lactation were also not accompanied by a negative burden on the organism of first-heifers. During this period, the average daily milk yield of Brown Swiss cows increased to the level of 28.1 kg, which was more than the first two months of lactation. That is, the level of feeding and the conditions of keeping contributed to the high physiological activity of the lactating organism of first-heifers.

After the first four months of lactation, the physiological activity of the udder of Swiss first-heifers decreased naturally. During the 6-month lactation period, the level of average daily milk yield of cows decreased to 26.8 kg, which fully corresponds to the physiology of lactation. This also applies to the 8-month period of the lactation function, when the level of milk productivity of animals decreased to an indicator of 23.2 kg. That is, during the activity of the lactating organism of cows, there was no suppression of milk secretion factors in the udder and a decrease in milk yield, which could be caused by the action of negative metabolic factors, which researchers McArt et al. (2012) and Zarrin et al. (2017).

Especially low milk yield was noted in the animal during the last two months of lactation, when the average daily productivity did not exceed 16.1 kg of milk. The decrease in milk yield during the period of 200 days of gestation in cows that lactated during standard lactation is also physiologically justified.

The researchers indicated that removing milk from cows' udders, especially with increased frequency of machine milking, significantly improves udder health (Hogeveen et al., 2000). The experimental cows were milked three times a day, and twice before starting in the dry period. Experimental studies have shown that cows at the beginning of lactation, which were milked more often, had an increase in the level of milk productivity (Hale et al., 2003; Dahl et al., 2004; Soberon et al., 2011). Moreover, even a week before the dry period, cows milked twice a day secreted more milk than cows milked once a day (Tucker et al., 2009). However, as other researchers have noted, lower milk yields before starting in the dry period may be beneficial for subsequent calving (Dingwell et al., 2004; Rajala-Schultz et al., 2005). And what is especially important is that first-heifers do not always respond to increased milking frequency by increasing milk yield (Abeni et al., 2005, 2008; Speroni et al., 2006). Researchers Jago and Kerrisk (2011) point to the high adaptive capacity of first-heifers, for example, that they better adapt to an automated milking system, unlike older cows. During the milking of first-heifers in the milking parlor, the stereotype of preparatory operations was maintained to induce a milk removal reflex, which was an important factor in maintaining the lactation function (Dzidic et al., 2004).

The entire lactation period of the animals was not accompanied by significant changes in milk quality, as indicated by the ratio of milk fat to protein, which was at the level of 1.13–1.17 units. As noted by Abeni et al. (2005), milk composition in terms of protein and fat content does not depend on the type of milking system.

More important for the concentration of milk fat in milk is the duration of the interval between milkings, as well as fluctuations in the milk yield (Bruckmaier et al., 2001). The level of energy supply and the organization of rest of the first-heifers contributed to a good adaptation to the conditions of the industrial complex with intensive exploitation technology. The fact that 68 % of their number lactated almost as long as standard lactation indicates the high adaptive plasticity of Swiss first-heifers. That is, these animals were characterized by a high reproductive function, since the service period was at the level of 117–118 days. There was no violation of the balance between the organism and the external environment in Swiss first-heifers with standard lactation, since the adaptation index does not exceed -3.7 units.

Only 32 % of Swiss first-heifers had prolonged lactation due to a long service period, which was 260 days in avarage. Buckley et al. (2003) indicate that a high level of milk production in the first third of lactation is associated with a reduced percentage of fertilization before the first pregnancy. Nevertheless, in cows with a prolonged lactation function, the balance between the organism and the external environment was not significantly disturbed, since the adaptation index was -11.8 units on average. Van Knegsel et al. (2013) note that lengthening the lactation period is a strategy for optimal use of genetic potential in combination with delayed fertilization. At the same time, some researchers indicate that there may not be a relationship between health disorders and the level of productivity of dairy cows (Fleischer et al., 2001).

Conclusion

In the conditions of a large industrial complex during the first lactation, Brown Swiss cows show high adaptability, which ensures the realization of milk productivity at the level of 8423.4–10132.6 kg with a milk yield index of 2124.0–2575.8 kg. At the same time, the rate of reaction of Swiss first-heifers in the interaction "genotype – environment" depending on the duration of the lactation period and the level of milk production, the adaptation index has a minor negative value, which ranges was assessed from 3.7 to 11.8 units.

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Authors' Contributions

LL and NK – data collection and processing; SP and IH – writing, reviewing and editing the article. We confirm that the manuscript has been read and approved by all named authors.

Conflict of Interests

The authors declare no conflict of interests. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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