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Effect of early weaning on behaviour, blood immunological parameters and performance in rabbits*

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Summary

The aim of the study was to assess the impact of the weaning date of young rabbits on their weight gain, plasma immunoglobulin levels and exploratory behavior. Animals, Termond White rabbits were divided into 2 groups: group I – rabbits weaned at 21 days of age (n = 10), group II – rabbits weaned at 35 days of age (n = 10). The material subjected to analysis was peripheral blood collected on 21, 35, 42, 84 days of life. On these days body weight was also measured. The immunoenzymatic method was used to determine the level of immunoglobulin class M and G. The exploratory examination was performed with open field test (OFT) on the 75th day of the rabbits' life. During the monitoring period, IgM levels were higher in group II of animals. The mean concentration of IgG was higher in animals from group I. The statistical analyses showed no significant differences between the Control and Experimental groups for IgM and for IgG. Also the differences between age groups were not significant for IgM and for IgG. In an experimental behavioral study, the statistical analysis showed no effect of weaning age on the number of fields explored by the young rabbits. Observing the trend of differences in body weight between animals from the groups, lower gains were shown in group I. However, statistical analysis does not confirm this trend.

Keywords: early weaning, rabbits, immunoglobulin, behaviour

The first task of a newborn rabbit is to find the mother, the nipple area on her body, and to grasp it and suck her in a coordinated manner. Meanwhile, the doe remains motionless at this point to facilitate this task for the young. These initial activities are of great behavioral and physiological importance to newborn mammals. The rabbits then perform the first articulation, coordinated with breathing and swallowing. The consumption of colostrum affects the arousal state of the young, thanks to which they learn the first social experiences after birth. The colostrum also provides the kits with high nutritional and immunoprotective properties through the initial hormonal response induced by milk consumption and the selection of the microflora of the young through contact with the mother's skin (4). Due to the fact that in rabbits and hares, females leave their offspring in the nest after giving birth and return to them only for feeding once a day for about 5 minutes, the first suckling of newborns is very important. If the suckling misses it, he will not be able to make up for the lack of nutrients resulting from the lack of absorption of colostrum, which may be associated with increased mortality in rabbits. Compared with cow and sow milk, rabbit milk is two and three times more concentrated in fat (12.7-18.9%) and protein (11.9-14.7%), respectively, and it contains one-third of the amount of lactose found in the other two milk types (1.0-1.9%) (1). About 47% of fat are medium-chain fatty acids (C8 and C10), which are bacteriostatic and contribute to the formation of the microflora of the digestive system of young rabbits (17). Lactation is the most energetically costly physiological process of the female reproductive cycle. During this period, the energy balance of the rabbit is usually negative and body reserves must be mobilized. (22). However, the literature data cited that young rabbits need colostrum and milk to adapt to the prevailing environment, acquire resistance to pathogenic microorganisms and learn appropriate

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behavior. In addition, during the lactation period, the doe has an impact on the development of caecal microflora in young rabbits (1). This is important because a high concentration of microorganisms in the digestive tract has a high impact on physiological, nutritional and immunological processes, and in herbivores it is crucial for the early adaptation of the young to fermentation and pathogen control. By the time of weaning, the caecal microflora is not sufficiently developed and stabilized, so there is a greater risk of intestinal infections. Hence, the risk of mortality is highest at weaning, and the most common cause of this is diarrhea, the etiology of which is multifactorial. Most often it is caused by colibacillosis, an infectious disease that is caused by the increased virulence of *Escherichia coli* bacteria. The second most common disease is coccidiosis caused by *Eimeria cocci* (6). The weaning time of young rabbits is a very critical moment in their life and also during the life of does. The weaning time can affect the health status and the growth performance of the young rabbits during the fattening period, particularly during the first post-weaning weeks. However, the weaning period also has a significant effect on the welfare and on the body condition of the does. By limiting the duration of the lactation and the rhythm of reproduction, the negative effects of litter rearing can be reduced and the reproductive usefulness of the female can be extended (2, 33). Negative energy balance during lactation negatively affects female fertility (12, 20), which is why the reproductive lifespan is shorter in intensively bred females than in extensively bred ones (22). New rabbit breeding systems try to combine appropriate female remating interval with early weaning of rabbits (33, 34). However, the time of weaning the rabbit should be optimized so as not to risk higher litter mortality.

The aim of the study was to assess the impact of the weaning date of young rabbits on their weight gain, plasma immunoglobulin levels and exploratory behavior.

Material and methods

The experiment was conducted upon receiving the permission granted from the Local Ethics Committee in Krakow (No. 37, 30 May 2016).

Animals. The experiment was conducted at the University of Agriculture in Krakow (Poland) in the Experimental Station of the Department of Genetics, Animal Breeding and Ethology. The research material consisted of Termond White rabbits. Rabbits were kept on bedding in wooden cages (80 cm × 70 cm × 65 cm) of dimensions in accordance with applicable standards. The cages, standing in an insulated hall (12 m × 25 m), were equipped with a water system (nipple drinkers) and with forced ventilation system. Animals were kept in a 14 L : 10 D light system (14 h light and 10 h darkness per day) with a light intensity of 60 lux. Average temperature on the farm varied from 10°C to 15°C, depending on the season. Humidity ranged between 50-60%. Animals had permanent access to water and complete granulated feed, and were under constant veterinary supervision. Cages and feeders were regularly cleaned and disinfected according to the animals' needs. In the experiment, we used 5 does and from each litter, one female and one male were randomly assigned to each group. The rabbits were weighed and vet checked to select animals of similar body weight and good health status for the groups.

Procedures. Animals were divided into 2 research groups, due to the time of weaning from the mother:

– group I (Experimental) – rabbits weaned at 21 days of age (n = 10),

– group II (Control) – rabbits weaned at 35 days of age (n = 10).

The material subjected to analysis was peripheral blood collected on 21, 35, 42, 84 days of life. Blood was collected from the marginal ear vein in the amount of 3 ml into tubes with coagulant EDTAk3. Plasma was obtained by centrifugation at 3,500 × g for 3 min.

Weight gain and veterinary status were assessed each time blood was collected. The following parameters were taken into account during the veterinary assessment: number of breaths per minute, body temperature and coat condition.

Determination of the level of immunoglobulin in the blood serum. The immunoenzymatic method was used to determine the level of immunoglobulin. Ready-made commercial kits from the manufacturer BT LAB E0148Rb for class M immunoglobulin and E0206Rb for class G immunoglobulin were used. The optical density of individual groups was measured in an ELISA reader equipped (Spectramax Plus 384, *Molecular Devices*) with an automatic data processing system. Duplicate wells were used for each samples. Plates were read using microplate reader set to 450 nm. Results were calculated based on standard curve. The sensitivity of the kit was 0.15 µg/ml.

Exploratory behavior. The exploratory examination was performed with open field test (OFT) on the 75th day of the rabbits' life. An open field area painted white from the inside, measuring 2.5 m × 2.5 m and 1 m high, was used to study the exploration of the environment, divided into peripheral squares (near the walls) and middle squares (central). Each animal's behavior was quantified by counting the number of times the animal passed through each square. A single open field square was considered as passed, in a situation in which all four limbs of the animal were in the given square. The duration of the observation was 3 minutes. The test field was cleaned with a 50% ethanol solution after each animal.

Behavioral patterns measured in the open field test (OFT) included:

• Number of squares examined – the frequency with which the rabbit crossed the grid lines with all four legs (a measure of motor activity), broken down into activity near the wall and activity in the center,

• Number of entries into the center field – the frequency with which the rabbit entered the center field with all four paws,

• Standing – the frequency with which the rabbit stands on its hind legs in the field,

• Defecation and urination – the frequency of defecation and urination during the test.

Statistical analysis. The traits analyzed were the body weight of the young rabbit, the immunoglobulin IgM and IgG level and the number of fields in the Open Field Test (OFT). The normality of the distribution was examined for all analyzed traits and a significant deviation from the normal distribution was found in each of them. For all traits the basic statistics (median, minimum and maximum, and number of observations) were estimated. The influence of group (control or experimental) and the age of young rabbits (21, 35, 42, 84 days of life) on the traits mentioned before were examined. To analyze the significance of the effect of group and young rabbits age on body weight and IgM and IgG levels, as well as to examine the effect of the housing system on the behavior of rabbits in the OFT a non-parametric one-way analysis of variance (NPAR1WAY procedure) with the Wilcoxon-Mann-Whitney test (S) was used. To examine the effect of age on body weight, the non-parametric one-way analysis of variance (NPAR1WAY procedure) with the Kruskal-Wallis test and, additionally, Dwass, Steel and Critchlow-Fligner (DSCF) method of testing for multiple comparisons between age groups was used. All statistical analyses were performed with using the SAS statistical package (28).

Results and discussion

Changes in body weight of rabbits. The body weight of rabbits weaned at 21 days of age ranged from 300 to 390 g (on average 348.08 ± 10.2 g). The health of the animals was very good. In the second group of the tested animals the rabbits were weaned from their mothers at the age of 35 days. The body weight of the animals from the first group ranged from 550 to 870 g (on average 680.83 ± 67.03 g), while in the second group from 620 to 1155 g (on average 907.50 ± 80.34 g). There were no significant differences between the Control and Experimental groups on the body weight at 35 days of life (S = 539.0; p-Value = 0.6355).

On the day of slaughter, on the 84th day of life, body weights were recorded at the following levels: from 1890 to 2750 g (on average 2173.33 g ± 144.43 g) in group I (Experimental); from 2506 to 3180 g (on average 2751.50 g ± 147.60 g) in the Control group (Tab. 1). Observing the trend of differences in body weight between animals from groups, lower gains were shown in group I. However, statistical analysis does not confirm this trend.

Tab. 1. The median, minimum and maximum of the body weight of rabbits in the different age groups

Age group (days of life)	Median	Minimum	Maximum	Kruskal-Wallis test value	Probability (significance)
21	357.5	300.0	390.0	40.92	< 0.0001
35	762.5	550.0	1155.0		
42	1637.5	1125.0	2180.0		
84	2383.0	1890.0	3180.0		

The impact of age on the body weight of young rabbits was highly significant (the Kruskal-Wallis test value of 40.92; p-Value < 0.0001). The multiple comparisons of DSCF test results between age groups of rabbits are shown in Table 2.

As shown in Table 2 all age groups differ significantly from each other, which means that young rabbits grew very quickly despite temporary health problems.

Tab. 2. The multiple comparisons of DSCF test results between age groups

Comparison	The DSCF value	P > DSCF
21 day and 35 day of life	5.8852	0.0002
21 day and 42 day of life	5.8852	0.0002
21day and 84 day of life	5.6045	0.0004
35 day and 42 day of life	5.7971	0.0002
35 day and 84 day of life	5.5966	0.0004
42 day and 84 day of life	4.6638	0.0054

Analysis of the concentration of immunoglobulin in the blood plasma. During the monitoring period, IgM immunoglobulin levels were higher in group II of animals (Control group). The average IgM results obtained in the study on the 42nd day of life of the rabbits were 323.43 µg/ml and 462.26 µg/ml, on the 84th day of life 284.09 µg/ml and 785.00 µg/ml for group I (Experimental) and for group II (Control), respectively. It should be emphasized that opposite trends of changes in the same period of life occurred in both groups. Namely, the mean concentration of IgM decreased in the group of rabbits weaned at 21 days of age, while it increased in the group of rabbits weaned at 35 days of age.

During the monitoring period, the mean concentration of immunoglobulin IgG class was higher in animals from group I (Experimental) on the 42nd day of life and amounted to 613.13 µg/ml. Then in this research group a decrease in the level of IgG was observed and on the 84th day of life the concentration was 471.78 µg/ml; this result was lower than for group II (Control group). In the Control group the reverse trend of changes in IgG concentration was observed compared to Experimental group I. The concentration of IgG immunoglobulin at 42 days of age was 518.13 µg/ml, then increased and reached the level of 940.93 µg/ml at 84 days of age. The median, maximum and minimum level of immunoglobulin IgM and IgG in the blood plasma of rabbits in the age classes for studied Control and Experimental groups are shown in Table 3. The median, maximum and minimum level of immunoglobulin IgM and IgG in the blood plasma of rabbits in the age classes are shown in Table 4.

The statistical analyses showed no significant differences between the Control and Experimental groups for IgM and for IgG (Tab. 3). Moreover, the differences between age groups were not significant for IgM and for IgG (Tab. 4).

Behavioral analysis. In the behavioral study, the open field test (OFT) examined the rabbits' level of

Tab. 3. The median, minimum and maximum level of immunoglobulin IgM and IgG in the blood plasma of rabbits for Control and Experimental groups

Immunoglobulin	Group	Median	Minimum	Maximum	Wilcoxon-Mann-Whitney test (S) value	Probability (significance)
IgM (μg/ml)	I[1]	246.160	85.00	682.22	136.0	0.1802
	II[2]	427.225	98.00	1923.33		
IgG (μg/ml)	I[1]	454.660	63.73	2083.94	124.0	0.5708
	II[2]	520.725	236.27	1859.59		

Explanations: [1] Group I (Experimental) – young rabbits weaned at 21 days of age; [2] Group II (Control) – young rabbits weaned at 35 days of age

Tab. 4. The median, minimum and maximum level of immunoglobulin IgM and IgG in the blood plasma of rabbits in the age classes

Immunoglobulin	Age class	Median	Minimum	Maximum	Wilcoxon-Mann-Whitney test (S) value	Probability (significance)
IgM (μg/ml)	42 day	341.670	85.00	1093.33	119.0	0.8212
	84 day	337.780	140.00	1923.33		
IgG (μg/ml)	42 day	304.145	63.73	2083.94	144.0	0.0567
	84 day	546.155	383.42	1859.59		

activity, anxiety, and willingness to explore new areas. In the conducted study, it was found that rabbits from group I (weaned on the 21st day of life) showed less willingness to explore a new environment. The average number of explored squares in this group was 5.4, while in group II (weaned at 35 days old) it was 11.17, in both groups the rabbits preferred to move around external fields. In the group I (Experimental) none of the rabbits reached the center of the field. In the group II (Control) one rabbit reached the center of the field within 18 seconds. Stand-ups were made by one rabbit from group I (number of stand-ups was 2) and three rabbits from group II (all with one stand-up). In both groups urination and defecation were not observed. The statistical analysis showed no effect of weaning age on the number of fields explored by the young rabbits (S = 19.5; p-Value = 0.0649).

In this study, the average body weight of rabbits from the Experimental and Control groups changed over time (in subsequent measurements). Similar preliminary results were obtained by Gallois (7). However, these authors observed that the group of earlier weaned rabbits matched their final weight with the animals in the control group. Also Ragab (27) has similar results, he observed early weaning had negative effects on growth traits during the fattening period, but these negative effects were compensated after 8 weeks of age. Taranto (30) found that the examined rabbits also showed significant differences in body weight between the groups up to the 42nd day of life, but as in Gallois (5), the final body weight in both groups did not differ significantly. Rabbits that were weaned earlier, i.e. on the 21st day of life, were not able to compensate for the lack of milk with solid feed, i.e. there were gaps in meeting the energy demand. This carries the risk of lower weight gains and, consequently, lower slaughter weight and carcass weight. McNitt and Moody Jr. (19) observed that, by day 28 the body weight of rabbits weaned on day 14 increased significantly slower than those weaned on day 28. These rabbits also reached the final slaughter weight 7 days later, which is consistent with our own observations. Furthermore, Kovács at al., El-Sabrout and Aggag (5, 15) found that rabbits weaned early (up to the age of 28 days) had significantly lower final body weight and achieved lower market prices than those weaned later (at 35 days of life). Early weaning can result in the stress of a sudden change of food. Young rabbits begin to eat solid food around 19 days of age. At 21 days of weaning age, they have not yet transitioned to solid food. Piattoni et al. (23) showed that rabbits weaned at 18 days of age did not eat for 1-2 days, then they suddenly started eating fodder. However, solid food does not compensate for the lack of milk, so early weaned kits gained a lower body weight at 32 days than those kept with their mother yet. In the study of Piattoni, the body weight of young rabbits weaned at 18 and 32 days of age equalized after about 50 days, contrary to the presented study where the final body weight of young rabbits differed significantly. Piattoni proved that the development of the rabbit cecum is affected by solid food and not milk. They weaned the young rabbits from its mother at 18 days of age, and over the next few days, they only observed a significantly greater growth of the cecum in these kits in comparison to other young rabbits who stayed with their mother all time. Additionally, Piattoni obtained similar weight gain results as in our study (8). Namely, rabbits weaned before 28 days of age gained weight much more slowly than other rabbits that stayed with their mother longer. It is also worth noting that the young rabbits that were fed exclusively with milk until the 28th day of life had noticeably lower weight gain. That indicates that the milk does not fully cover the needs of the young since the 22nd day of life and they can’t reach normal body size. McNitt and Moody Jr. observed increased mortality in the young weaned at 14 days of age compared to those weaned at 28 days. These authors observed that some litters are more susceptible to mortality because of the strong influence of birth weight (19). Heavier individuals have a better chance of survival, therefore larger litters should not be weaned early as their average body weight is lower compared to smaller litters.

In addition, the survival of young is affected by pathogens. The young rabbits weaned at 14 days are much more susceptible to, for example, enteritis, due to the elimination of the antimicrobial benefits of milk, than have those kept with their mother longer. The advantage of early weaning the young from the mother is the possibility of choosing the right solid fodder for young rabbits. When the young are kept with the female, the fodder is matched to the mother's needs, i.e. with a focus on high energy content. Gidenne and Fortun-Lamothe (8) showed that the requirements of the rabbits of up to 35 days of age are antagonistic to the nursing mother. The young between 28 and 35 days of age need more fiber and less starch in their fodder, unlike the mother, who has a high energy requirement. Therefore, adequate nutritional requirements can lead to improved rabbit breeding, but further research is needed to explore this topic.

Birth weight and final weight of young rabbits, as well as litter size, are also influenced by production intensity (21). The time at which the female will be mated after giving birth and the age at which the young rabbits will be weaned determines how much time the female rabbit's body has for regeneration. Nicodemus showed that in intensive production, where the young are weaned before 25 days of age, rabbits had limited digestive capacity, but they managed to compensate for the deficiencies in the diet with highly digestible food (21). Our results, as well as the cited studies of other scientists, show that it is possible to improve rabbit breeding, body weight gain and slaughter efficiency. However, further research in this direction is needed. Particular attention should be paid to the selection of an appropriate diet for early weaned rabbits to compensate for deficiencies associated with the lack of milk. It is also worth analyzing the situation when there is a stress associated with a sudden change of food. The female should not be forgotten either; improving the rhythm of intensive production can significantly affect the size of litters, the number of litters per year or weight gain of young rabbits.

The antibody repertoire of rabbits has been of interest to immunologists for decades, partly because of the ease with which large amounts of high-affinity antibodies can be obtained in serum, and partly because of the presence of different genetic variants. The rabbit is one of the few known vertebrate species in which the use of combined splicing of multiple VH, DH gene segments during immunoglobulin heavy chain gene rearrangement is limited. The rabbit immune system is of interest to researchers due to the exploitation of both gene conversion and the role that gut-associated lymphoid tissues (GALT) and gut flora play in the development of the primary antibody repertoire. However, there are no literature reports related to the influence of the weaning period on the level of immunoglobulin. The synthesis of endogenous immunoglobulin in rabbits begins between 4 and 8 weeks after birth (16). In our research, this process can be easily observed in animals from the control group, in which the level of immunoglobulin, both IgG and IgM, decreased by the 42nd day of life, and in the following days the concentration of the tested antibodies increased. It can be assumed that the decrease in the level of immunoglobulin was the result of the depletion of the pool of maternal antibodies, and the observed increase is the result of endogenous production. In our studies, large individual differences in the level of immunoglobulin were observed. Similar observations are described by Chappuis (3) with differences between littermates and between litters, the highest level of immunoglobulin in the blood of piglets, puppies and kittens were described after 36-48 hours after birth. Since this moment, a gradual decline in maternal antibodies begins. Their level reaches 1-3% of their original value after 60 days in piglets, after 40 days in lambs, after 100 days in calves, after 115 days in foals, after 30 days in puppies and kittens, which is also visible in our research. Irrespective of the presented data, the duration of maternal immunity in a newborn is difficult to determine precisely, because it depends on many factors, both from the mother's side and environmental factors, including exposure to pathogens (32). In the animals of the experimental group, in our research, this process is not visible and from the day of weaning, an increase in the level of IgM is observed. It seems that the obtained results may be significantly influenced not by the fact of weaning and switching exclusively to solid fodder, but by the resulting changes in the intestinal microflora.

The open field test, in addition to the level of fear of the stress factor, which is open space, determines locomotor activity and the degree of exploration in a new, hostile environment (9). In our own research it was not found that the age of weaning had a significant effect on the number of fields explored by young rabbits. In order to study exploratory behavior, the open field test was performed, which is based on the natural tendency of animals to avoid a new environment on the one hand, and to explore it on the other (9). In our research, defecation and urination were not observed in both groups. Here, the interpretation of the frequency of defecation and urination is controversial. Some scientists say that an increase in defecation indicates increased anxiety. Other scientists disagree and argue that defecation and urination are signs of emotionality but cannot be attributed to anxiety (24-26, 31). Weaning is a stressful situation for all domestic animals. To reduce stress, weaned rabbits should be housed in groups created from each litters or mixed groups of animals of the same age (10). Studies show that rabbits should be weaned at 4-6 weeks of age. The ideal solution is to move the mother to another cage and leave the young rabbits in the original cage where they lived until weaning, although this method is rarely used in breeding conditions. Unfortunately, in intensive farming rabbits are weaned at the age of 21 days for economic reasons (11). Post-weaning behavioral studies of rabbits indicate that rabbits weaned at an early age (21 days) tend to stay in groups. Matics et al. (18) observed (in their free-choice system) that rabbits prefer the company of other animals to solitude after weaning, additionally the rabbits chose small cages. A study by Zotte et al. (35) showed that when rabbits were given a choice between a mirror cage and a mirrorless cage, 72% of the animals chose the mirror cage. Kazimierczak et al. (13) observed that weaned rabbits did not show agonistic behavior due to their young age (before puberty). Rabbits (in all groups) showed preferences for companionship and location. In addition, these authors tried to associate various forms of behavior, e.g. correlations between exploratory and affiliative behaviors and other individual and locomotor behaviors were highly significant. Simitzis et al. (29) did not observe the influence of maternal condition during the prenatal period on the behavior of young rabbits in the open field test. Early weaning from the mother can not only affect social behavior but also affect the response to stimuli. Studies in mice by Kikusui et al. (14) suggest that disruption of the mother-infant bond by early weaning impairs pain transmission and modulates pain sensitivity.

Although there was no statistically significant effect of early weaning rabbits on body weight, immunoglobulin levels or behavior in this experiment, negative effects of early weaning could be seen, especially in the changes of body weight gain and immunoglobulin levels. The period from birth to weaning is characterized by a particularly high mortality rate of farm animals, which results in significant economic and breeding losses. Therefore, it is advisable to conduct research to deepen the knowledge about the health of young rabbits and to select the optimal weaning time to minimize losses caused by the death of young animals.

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