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Effect of divergent selection for total body fat content on condition and productive performance of rabbits

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1. BACKGROUND OF RESEARCH, OBJECTIVES

At the end of pregnancy and at the peak of lactation, rabbit does are unable to consume enough feed to cover their energy needs, so they are forced to mobilise their own reserves, which can lead to a deterioration in their condition. At the time of the first insemination, the body weight of rabbit does reaches 75-80% of their adult weight. During the first pregnancy and then lactation, rabbit does also gain weight, which also requires energy and nutrient intake. In young rabbit does, this multiple energy deficiency state (she is nursing, pregnant with the next litter and her own body is also growing) can lead to a serious deterioration in condition, poorer production results and often culling. Condition can be characterised by the amount of fat depots, which can be most accurately determined by chemical analysis of the whole body after a test slaughter. Among the methods that can be performed on live animals, ultrasound and total body electrical conductivity (TOBEC) are less reliable, while magnetic resonance imaging provides significantly more accurate information, but is expensive and time-consuming, which does not allow their use for selection purposes. In contrast, computed tomography (CT) is fast, accurate, and provides a reliable picture of the composition and fat content of the whole body, and last but not least, it has a good price/value ratio.

In addition, consumers in developed countries are increasingly demanding the quality of the food they eat. As a result, the meat industry also places great emphasis on meat quality. Fat content significantly affects the quality of meat.

1.1. Objectives

Based on the above, our goal was to examine the possibility of improving condition through selection based on CT examinations and what effect this has on the production of rabbit does. We wanted to answer the above question by means of divergent selection, i.e. by parallel selection of fatter and leaner lines, and by examining the production of the rabbit does selected in this way.

Since rabbits are usually chosen for selection purposes around the age of 10 weeks, their first insemination takes place only significantly later, after the age of 16 weeks, so we thought it important to find out the relationship between the total body fat content estimated before the first insemination (at 16-19 weeks of age) and the body fat content estimated at the age of 10 weeks.

The primary goal of rabbit breeding is meat production and improving the meat quality, so our additional goal was to investigate how divergent selection for total body fat content affects the production and slaughter characteristics of growing rabbits.

2. MATERIAL AND METHOD

2.1. Divergent selection

A divergent selection was performed over four generations — between 2014 and 2016— based on the Pannon Ka rabbit breed, which is one of the maternal rabbit breeds of the Pannon Rabbit Breeding Programme at the Kaposvár University. The development of the Pannon Ka breed commenced in 1999 and it was officially recognised as a synthetic breed in 2003. The breed has been selected for number of kits born ever since.

At the beginning of the study, 351 Pannon Ka female rabbits were randomly selected from two consecutive litters in November 2013 and January 2014. The selection trait was the fat index, which was calculated by dividing the volume of fat content estimated by whole body CT (cm^3) by the live weight (kg). CT examinations were performed using a Siemens Sensation Cardiac CT machine at the Institute of Diagnostic and Oncoradiology, University of Kaposvár, using the following settings: tube voltage – 120 kV, radiation dose 140 mAs, spiral data acquisition mode, pitch factor 1, field of view 500 mm. Cross-sectional digital imaging was performed in accordance with the ISO 9001:2015 quality management system and the ISO 14001:2015 environmental management system. During the recording and data collection, the rabbits were restrained in a prone position in special holders with straps, without anaesthesia, in triplets. From the raw data, overlapping images were reconstructed with a slice thickness of 2 mm, which were archived in “dicom” (Digital Imaging Communication Of Medicine) format.

The three individuals were automatically separated from the image sequences, and then the holders were removed from the images using an algorithm developed for this purpose (Kovács et al., 2013). To determine the fat volume, we used a thresholding technique using OpenIP software (Kovács et al., 2010), where voxels with Hounsfield units between -20 and -200 (Romvári et al., 1996; McEvoy et al., 2008) were segmented as foreground, collecting data from the entire body. The reduction of the partial volume effect was absolutely necessary, since the mixed voxels, especially in the skin-air boundary, resulted in large volumes with a density characteristic of fat. A morphological tool (erosion) was used for this task (morphological correction) (Beier et al., 1998; Kovács et al., 2010, Donkó et al., 2016). The volume of the anterior voxels selected after the corrected segmentation was further used to calculate the fat index.

To avoid inbreeding, the future breeding animals were selected in equal proportion from the 4 lines of the original breed. In order to be able to select breeding rabbits from as large a population as possible, we chose breeding animals from two consecutive litters for the 3rd and 4th generations. For the next generation, we selected male rabbits from the first litters and female rabbits from the second litters, so that the bucks were six weeks older than the does, so that there would be sufficient and good quality sperm at the first insemination (Gen2). We proceeded similarly when selecting the next generation (Gen3). For the purpose of optimize the costs of CT examinations, we usually selected one male and one or two female rabbits with a body weight close to the average (1.88-2.80 kg) from the littermates. In total, the study was based on the fat index records of 1663 Pannon Ka rabbits, the pedigree included 2668 individuals. During the several generations examined, we had to select from populations with very different average weights during each selection, which is mainly the reason for the wide body weight range. Since we followed the results of 4 litters in each generation, the rearing period of the breeding rabbits of the following generations fell on different seasons, which significantly affected the body weight of the rabbits.

The female rabbits in the first 3 generations were first inseminated at the age of 19 weeks in accordance with the farm practice, however, the difference in body fat content may be more important when insemination happens at an earlier age. Therefore, the female rabbits of the 4th generation were taken into breeding at 16.5 weeks of age to see how the production of Fat and Lean rabbits develops if we inseminate them as early as possible, according to the most widely used method. In rabbits, the selection of future breeding animals is usually done at 10 weeks of age, because they reach slaughter weight (min. 2.5 kg) at 10-11 weeks of age and individuals not selected for further breeding are sold. Therefore, CT examinations for selection must be performed at 10 weeks of age at the latest. It is important to mention here that fat accumulation in rabbits becomes very intense after 10 weeks of age (Szendrő et al., 1998), so by the time of breeding (16-20 weeks of age), the body fat content of rabbits changes significantly. Therefore, the female rabbits selected for breeding were examined again with CT before the first insemination.

2.2. Housing condition

The animals were kept in the rabbit farm of the University of Kaposvár, where the daily light was 16 hours, and the temperature – depending on the season – varied between 15 and 28 °C. The rabbits were allowed to consume commercially available feed *ad libitum* and drink unlimitedly from nipple drinkers. A 42-day reproduction rhythm was applied, fresh, diluted, individual sperm was used for inseminations, all Fat does were inseminated with the sperm of Fat and all Lean does with the sperm of Lean buck. Cross-fostering was used within the group (1st litter: max. 8 rabbits/litter, for subsequent litters max. 10 rabbits/litter), free nursing was used.

The results of the first 4 inseminations were evaluated in each generation. The kindling rate, the body weight of the doe at kindling, the litter size (total, born alive and stillborn, after litter equalization, 21 and 35 days old), the litter weight at 21 and 35 days old, and the individual body weight at 21 and 35 days old, and the suckling mortality between 0-21 and 0-35 days were calculated. From the total results of the four inseminations, the number of weaned rabbits per 100 inseminations and the total weight of weaned rabbits were calculated. A fattening experiment was performed with male rabbits from the 3rd litter of each generation. At 35 days old, individuals representing the average liveweight were randomly chosen (60 Lean and 60 Fat rabbits). The animals were housed in wire-mesh cages (3 rabbits/cage; 16 rabbits/m^2).

The weight and feed intake of the rabbits were measured weekly between 5 and 11 weeks of age. Daily weight gain, daily feed intake and feed conversion were calculated. Mortality was recorded daily. Body weight and weight gain were assessed individually, and feed intake and feed conversion were assessed per cage.

2.3. Statistical analysis

Pearson correlation coefficient and partial correlation were calculated between the scapular fat and perirenal fat and the total body fat volume estimated based on the processing of CT images, and t-test was used to compare the means of the groups. The statistical analysis was performed using the R statistical package version 3.6.1 (R Core Team, 2013).

Heritabilities, breeding values and genetic trends of fat indices were estimated applying best linear unbiased predictor (BLUP) and restricted maximum likelihood (REML) methods, using PEST (Groeneveld, 1990) and VCE6 software (Groeneveld et al., 2008) respectively. The fat indices of the Lean and Fat lines were considered as the same trait, thus a single trait animal model was applied. The structure of the model for fat index was the following:

$$y=Xb+Za+Wc+e$$

where y=vector of phenotypic observations, b=vector of fixed effects, a=vector of additive genetic effects, c=vector of common litter effects, e=vector of residuals, X, Z and W are incidence matrices linking phenotypic records to parameters. Residuals were normally distributed with mean zero and they were uncorrelated. Additive effects were normally distributed with zero mean and a variance covariance structure defined by the relationship matrix.

Additive, common litter effects and residual effects were uncorrelated. The fixed effects taken into consideration were sex (males and females), line (Lean and Fat) and year-season respectively.

The relationship between 10-week-old and pre-insemination fat indices was assessed using Spearman rank correlation coefficient. The analysis was performed using R software (ver.3.6.0) (R Core Team, 2018) and ggplot2 (ver.3.2.0) (Wickham, 2016).

The productive traits of the does per generation were analysed using GLM tests (fixed effects were line (Lean or Fat) and insemination order (1-4)) using R software (ver.3.6.0) (R Core Team, 2018). Kindling rate and mortality were assessed using Chi-square tests.

When evaluating the productive and slaughter traits of growing rabbits, the effects of the direction of selection (Lean, Fat) and the successive generations (1-4), as well as the interaction between the two, were tested using the SAS GLM procedure. (Since we did not obtain a significant interaction between the factors, the results of these are not reported). The least squares difference analysis was performed using the Tukey test (pdiff option of the GLM procedure). Mortality data were evaluated using the Chi-square test.

3. RESULTS

3.1. The effectiveness of estimating total body fat content in growing rabbits using computed tomography

In our study, the condition of the rabbits was expressed as the total volume of the fat tissues of the whole body measured by CT. As a reference, we used the weight of the two largest fat depots (scapular and perirenal), which were measured during test slaughter. The parameters measured in the two groups (Fat and Lean) were shown separately and together, and their means were compared. The live weight of the individuals in the two groups didnot differ from each other. The amount of scapular fat was on average one third of the perirenal fat in both groups. The scapular and perirenal fat content of the Fat group was approximately 1.5-2 times higher, than that of the Lean group. These values support the effectiveness of selection for fat content, similar to the results obtained in previous generations.

In the fifth generation of divergent selection for total body fat content, the perirenal and scapular fat depots of the carcass were combined and compared with the fat volume estimated by whole body CT. The fat volume results obtained by fully automatic image processing, supplemented with the thresholding technique and morphological correction, gave a medium to close correlation with the weight of the fat depots. The correlations corrected for body weight were moderate in the case of the Lean rabbits and showed a strong correlation in the Fat group and the two fat depots (scapular and perirenal), and when the two groups were combined ($R=0.749$-0.917; $P<0.01$). Correction by body weight resulted in a decrease of the correlation coefficient in the Lean group by approximately one tenth. The decrease was more moderate in the Fat group, while when the two groups were evaluated together, the decrease was 0.01–0.03. The results suggest that the condition determination was burdened with a greater error in the case of lower fat content (Lean group), and in such cases the effect of live weight is more significant.

3.2. Divergent selection for fat index in Pannon ka rabbits: genetic parameters, selection response

The estimated heritability was moderate (0.28±0.03), while the magnitude of the common litter effect was low (0.10±0.02). Heritability estimates for fat related traits are not too frequent in the literature because, unless non-invasive techniques such as CT or total body electrical conductivity (TOBEC) are used, estimating heritability requires slaughtering animals. The females had on average 2.1 cm^3/kg higher fat index than that of the males, while year-season effects were highly variable, showing a maximum difference of 17.8 cm^3/kg between the best and worst year-season. When the seasonal and sex effects were removed, the estimated breeding values (averaged per generation) gave a much smoother trend. The genetic improvement achieved during this study roughly corresponded to the additive genetic standard deviation (9.6 cm^3/kg) of the evaluated dataset. The intensity of selection for fat index in the 2^{nd} generation can be said to be relatively low, as in some cases, in order to avoid inbreeding and maintain the minimum number of breeding animals, individuals from each line had to be chosen for further breeding that did not have a sufficiently low fat index in the Lean group or a sufficiently high fat index in the Fat group.

3.3. Examination of the relationship between total body fat content estimated at 10 weeks of age and before the 1st insemination

A weak correlation was found between the fat indices calculated at 10 weeks of age and those calculated before the first insemination of the female rabbits selected for breeding in the 1st and 2nd generations ($R^2 = 0.325$ and 0.260, respectively; $P<0.001$). In the 3rd and 4th generations, a moderate correlation was found ($R^2 = 0.548$ and 0.669, respectively; $P<0.001$). In the 4th generation, as a result of the above and the younger age at the 1st insemination (16.5 weeks), an even stronger correlation was found between the fat indices estimated at 10 weeks of age and those estimated before 1st insemination. In the first three generations, due to the older age at 1st insemination (19 weeks), the condition of even less fatty individuals at 10 weeks of age may have improved, so that the difference in fat indices between groups at 1st insemination was no longer as great as at selection. By excluding individuals with “outlier” values from further breeding, the difference between groups and the accuracy of fat index estimates at the two time points can be increased. It may therefore be worthwhile to carry out the selection in two steps, in such a way that the “outlier” individuals are excluded based on the body fat content re-estimated before 1st insemination.

3.4. Effect of divergent selection for total body fat content on the performance of rabbit does

Based on the combined data of the 1^{st}-4^{th} inseminations, the kindling rate reached a high level in both groups. In the first three generations, we did not find any statistically proven differences between the groups, however, in the fourth generation - where the does were 1^{st} inseminated at 16.5 weeks - the Fat group kindled significantly more kits than the Lean group (70.1% vs. 85.2%; $P<0.001$). In the case of the later breeding age of 19 weeks used in generations 1-3, the Lean rabbits could also reach a suitable condition. In the first two generations, we did not find any differences in the does' body weight at kindling, but in the third generation, the Lean rabbits were heavier than the Fat does (4.20kg vs. 4.11kg; $P<0.05$), and this difference increased further in the fourth generation.

There was no difference between the two groups in the number of kits total, born alive and stillborn, 21- and 35-day-old kits in any generation. In the first two generations, there was no difference in the individual body weight of the kits at 21 and 35 days, but in the third generation, the kits of the Lean mothers reached a significantly higher weight (21 days: 352g vs. 331g; 35 days: 832g vs. 799g; $P=0.001$). In the fourth generation, however, no difference was found between the groups in the weight of the kits at 21 and 35 days. A significant difference was found in the suckling mortality between birth and 21 days of age only in the fourth generation (13.3% vs. 9.9%; $P<0.01$), with more kits died in the Lean group than in the Fat group. From the point of view of farmers, it is important to know how many rabbits can be weaned per given number of inseminations and at what body weight. In the number of weaned rabbits per 100 inseminations, in the 1st, 2nd, 3rd and 4th generations, we obtained a difference of 4; 1; 2 and 24% in favour of the Fat rabbits, respectively. In the total weight of weaned rabbits per 100 inseminations, in the first generation, we obtained a difference of 4% between the groups in favour of the Fat rabbits, this difference decreased to 1% in the 2nd generation, and then in the 3rd generation, we were able to wean rabbits with a total weight that was almost 6% higher in the case of the Lean rabbits. The latter is probably due to the fact that, unlike the other generations, the kindling rate of the does was almost the same, while the weight of the Lean rabbits was higher at 35 days of age. In the 4th generation, however, we were able to wean 24% fewer rabbits from the Lean does.

3.5. Effect of divergent selection for total body fat content on the performance and slaughter traits of growing rabbits

In the 1st and 2nd generations, there was no difference in the body weight of the rabbits at either 5 or 11 weeks of age. In the 3rd generation, rabbits selected for low body fat content were heavier at 5 weeks of age than the Fat rabbits (841g vs. 812g; $P<0.05$). We did not find any significant difference in weight gain or body weight at 11 weeks in either generation. There was no difference in feed intake between the lines in the first two generations, but in the 3rd and 4th generations, the Fat rabbits consumed 5% more feed than the Lean rabbits ($P<0.05$). Mortality was not affected by the direction of selection in either generation.

There was no difference in dressing out percentage between the groups within each generation, and as selection progressed, dressing out percentage increased in both groups. In the proportion of the fore part compared to the reference carcass, a significant difference was obtained in the 2nd and 4th generations in favour of the Lean group. In the 1st generation, the proportion of the mid part was higher in the Fat group, but in the later generations, no such difference was found. The proportion of the hind part did not differ in the two groups in the first two generations, but in the 3rd and 4th generations, the proportion of the hind part was higher in the Fat rabbits than in the Lean ones. As a result of the divergent selection for total body fat content, the perirenal fat and the scapular fat was higher in the Fat group. In our study, due to the change in feed composition and the effect of different seasons, the symmetry of the divergent selection cannot be reliably assessed. When the rabbits were fattened and slaughtered in the summer, feed intake and fat deposition decreased compared to other seasons. The ratio of perirenal fat and scapular fat compared to the reference carcass was 1.74, 1.57 and 1.69 times higher in the Fat line than in the Lean line.

In the 1st generation, the composition of the four meat parts was nearly identical in the Lean and Fat rabbits ($P>0.05$). When comparing the Lean and Fat groups within a generation, the fat content of the *Longissimus dorsi* muscle was similar ($P > 0.05$) in the two groups in all four generations. A similar result was observed for the meat on hind legs in the first two generations, but in 3rd generation of the divergent selection, the fat content showed a different trend ($P<0.10$) in the group selected for higher fat content than in the Lean group. The difference became significant ($P<0.001$) in 4th generation, where the Fat group had a higher fat content than the Lean group (5.98 vs. 5.10 g/100 g meat in the Fat and Lean groups). In the case of forelegs and abdominal walls, the difference between the two groups in terms of meat composition was already evident in the 2nd generation and the difference was maintained in the following generations. However, the amount of fat was not the only parameter affected by the divergent selection for total body fat content. The increase/decrease in fat content was accompanied by an opposite change in moisture content ($P<0.001$), while the protein content remained unchanged ($P>0.05$). The only exception was observed in the foreleg samples of the 4th generation: together with moisture and fat content, the protein content also differed in the Lean and Fat groups (16.2 vs. 15.2 g/100 g meat in the Lean and Fat groups; $P<0.01$).

The effect of generation was significant ($P<0.05$) for all meat parts we examined, which is due to progressive and cumulative changes in the composition of each meat part over generations of divergent selection, regardless of the direction of selection. The strong effect of generation indicates that the chemical composition of the meat parts changed over the four generations, but this effect includes seasonal/group bias. The difference between the two lines in all meat parts increased from generation to generation. The differences in fat content between generations 1 to 4 in the four cuts were: 0.12-0.16-0.23-0.19% in the *Longissimus dorsi* muscle, 0.41-0.58-0.67-0.85% in the hind legs, 0.32-2.56-1.97-3.86% in the forelegs, and 1.41-2.91-3.16-3.47% in the abdominal wall. However, a significant effect was only observed in the fat content of the foreleg in generations 1 and 4 ($P<0.001$). No significant differences were observed in the other cuts due to the relatively limited sample size and variability of the data.

4. CONCLUSIONS AND SUGGESTIONS

A significant difference was found between the Lean and Fat rabbits in the total body fat tissue volume, the weight of the scapular and perirenal fat determined by CT, which proves the efficiency of the selection. Based on the strong correlation and partial correlation values between the scapular and perirenal fat and the total body fat content determined by CT, the developed CT examination and evaluation method (elimination, morphological correction) can be used in all types of selection or experimental work in which the fat content of live rabbits needs to be estimated. The divergent selection experiment aimed at establishing the Fat and Lean rabbit line was successful, the difference between the body fat content of the two lines increased from generation to generation.

According to the results obtained, the selection method based on 10-week-old fat indices is suitable for selecting rabbits intended for further breeding. The effectiveness of the method can be increased if a new estimation is possible before the 1st insemination, based on which the breeding of individuals with an "outlier" value can be prevented.

Based on the results of the first four generations, there is no clear difference, but the kindling rate of does selected for high body fat content was more favourable when the does were inseminated at a younger age.

Selection for lower total body fat content may be beneficial for the sire lines, thus producing meat-producing rabbits with better feed conversion and lower slaughter losses. On the other hand, selection for higher total body fat content may be advantageous for the does, thus providing them with a larger energy reserve that they can mobilize when needed, i.e. at the end of pregnancy or near the peak of lactation.

In general, the results confirm that the effectiveness of the divergent selection for total body fat content in the Lean and Fat lines was also reflected in the fat content of the examined meat parts.

5. NEW SCIENTIFIC RESULTS

1. I have demonstrated that the CT examination and evaluation method developed by us can be used in selection or experimental work in which the body fat content of live rabbits needs to be estimated. The fat volume values estimated based on CT images, using fully automatic image processing, the thresholding technique supplemented with morphological correction, showed a moderate to strong correlation ($R = 0.749$-0.917; $P<0.01$) and a moderate to strong partial correlation corrected for live weight ($R = 0.650$-0.907; $P<0.01$) with the weight of individual fat depots of rabbits measured at slaughter.

2. I found that selection based on fat indices estimated at 10 weeks of age (total body fat content estimated based on CT examination in relation to body weight) is suitable for selecting rabbits for further breeding. In the 3^{rd} and 4^{th} generations, a moderate correlation ($R^2 = 0.548$ and 0.669, respectively; $P<0.001$) was found between the fat index values estimated at 10 weeks of age and those estimated based on CT examination performed before the first insemination. However, it would be worthwhile to perform the selection in two steps in such a way that individuals showing unfavourable values are excluded based on the fat content estimated before 1^{st} insemination.

3. The heritability of the fat index estimated during selection was moderate ($h^2 = 0.28 \pm 0.03$).

4. The number of rabbits weaned from does selected for higher body fat content based on the total body fat content of the rabbits estimated by CT was higher in each generation than the number of rabbits selected from does with low body fat content.
5. As a result of selection based on the total body fat content of rabbits estimated by CT, the feed conversion ratio of growing rabbits of the line selected for increasing body fat content were less favourable in the 4th generation than in the line selected for decreasing body fat content (7.7%; $P<0.05$). The ratio of perirenal fat to the reference carcass was significantly higher in the line selected for a higher fat index than in rabbits selected for decreasing fat index (1.79% vs. 0.88%; $P<0.05$).
6. The result of the divergent selection for total body fat content was also reflected in the development of fat depots and fat content of different meat parts of the growing rabbits. The weight of scapular and perirenal fat and the fat content of various meat parts of rabbits selected to reduce total body fat content were lower in all generations than the weight of scapular and perirenal fat and the fat content of various meat parts of rabbits selected to increase total body fat content.

6. PUBLICATIONS ON THE TOPIC OF DISSERTATION

6.1. Peer-reviewed papers published in foreign scientific journals

Kasza, R., Matics, Zs., Gerencsér, Zs., Donkó, T., Radnai, I., Szendrő, Zs., Nagy, I. 2020. Divergent selection for fat index in Pannon Ka rabbits: genetic parameters, selection response World Rabbit Science 28: 3 pp. 129-133.

Kasza, R, Donkó, T., Matics, Zs., Nagy, I., Csóka Á., Kovács Gy., Gerencsér Zs., Dalle Zotte A., Cullere M., Szendrő Zs., 2020. Rabbit Lines Divergently Selected for Total Body Fat Content: Correlated Responses on Growth Performance and Carcass Traits, Animals, 10(10), 1815; doi:10.3390/ani10101815

Cullere, M, Szendrő, Zs, Matics, Zs, Gerencsér, Zs, Kasza, R, Donkó, T, Dalle Zotte, A, 2022. Rabbits Divergently Selected for Total Body Fat Content: Changes in Proximate Composition and Fatty Acids of Different Meat Portions. ANIMALS, 12: 18 p. 2396

6.2. Peer-reviewed papers published in Hungarian scientific journals

Kasza, R, Szendrő, Zs., Matics, Zs., Gerencsér, Z, Nagy, I, Kovács, Gy, Csóka, Á, Petneházy, Ö, Garamvölgyi, R, Repa, I, Donkó T., 2021. Növendéknyulak teljes test zsírtartalmának becslése computer tomográffal (Estimation of total body fat content in growing rabbits by means of computed tomography) ÁLLATTENYÉSZTÉS ÉS TAKARMÁNYOZÁS 70 : 1 pp. 47-57. , 10 p

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