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Original article

Non-invasive study of changes in body composition in rabbits during pregnancy using X-ray computerized tomography

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Abstract — The objectives of this study were: 1) to follow changes in body composition during pregnancy using a non-invasive method; 2) to compare the results obtained with X-ray computerized tomography (CT) and with the slaughter method; and 3) to study the influence of litter size on changes in body reserves in pregnant does. Seventeen pregnant Pannon White rabbit does were examined by means of CT on the day of insemination, on days 14, 21 and 28 of pregnancy, and a few hours after parturition. At comparable time points, non-pregnant does ($n = 26$) were also scanned as a control group. During the CT procedure 27 images (scans) were taken from all of the animals in the body region from the scapular arch to the femoral-tibial articulation. The scans were processed by means of the computerized imaging technique, to obtain three-dimensional (3D) histograms that represented the changes in the water content, and in the fat and muscle tissues of the does. On the day after parturition all the does were slaughtered and the fat content of their empty bodies was subjected to chemical analysis. In the first 14 days of pregnancy little change was detected in body composition. Thereafter, in the abdominal region a marked increase was noted in densities corresponding to water and water-rich tissues, in correspondence with the growth of uterine contents. From day 21 of pregnancy a decrease in fat was noted in the pregnant does and an increase in the same reserves in the non-pregnant control group. To compare the fat content of animals in different physiological states so-termed fat indices were created by relating pixels corresponding to fat densities to the total number of pixels. In the same way muscle indices were created to demonstrate the changes in the muscle reserves during the experimental period. To test the accuracy of estimation by fat indices a regression analysis was carried out between the index numbers and the fat content subjected to chemical analysis. The result of this analysis showed a high correlation between the para-

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meters examined. The same fat indices demonstrated a higher mobilisation of body reserves in does with higher litter size than in those with smaller litters.

X-ray tomography / body composition / pregnancy / rabbit / reproduction

Résumé — Étude de l'évolution de la composition corporelle au cours de la gestation chez la lapine, à l'aide d'une méthode non invasive : la tomographie aux rayons X assistée par ordinateur. La tomographie assistée par ordinateur (CT) a été utilisée pour : 1) déterminer la variation de la composition corporelle des lapines durant leur gestation ; 2) comparer les résultats obtenus à l'aide de la CT et avec la méthode de l'abattage comparatif ; 3) étudier l'influence de la taille de la portée sur l'évolution des réserves corporelles pendant la gestation. Dix-sept lapines ont été explorées à cinq moments (insémination, 14, 21 et 28 j de gestation et quelques heures après la mise bas). Vingt-six femelles non gestantes (NP) ont été examinées en même temps, comme groupe de référence. Toutes les femelles ont été sacrifiées le lendemain de la mise bas. Pendant les premiers 14 j de gestation, les changements de composition corporelle ont été très faibles. Toutefois, au niveau de la région abdominale une augmentation de densité a été observée, correspondant à la croissance des tissus utérins et des fœtus. À partir du 21^{e} jour de gestation, les réserves corporelles lipidiques diminuent chez les femelles gestantes tandis que pendant cette même période elles augmentent chez les femelles non gravides. Pour comparer les quantités des tissus adipeux et musculaire chez les femelles soumises aux différents états physiologiques, des « Indices du gras » et des « Indices musculaires » ont été créés. L'analyse de régression a mis en évidence une étroite corrélation entre ces indices et les contenus du gras analysé par voie classique. Les mêmes Indices ont démontré que plus les femelles ont une portée de grande taille, plus la mobilisation de leurs réserves corporelles est forte.

tomographie aux rayons X / composition corporelle / gestation / lapin / reproduction

1. INTRODUCTION

It is widely known that the body composition of rabbit does is markedly affected by pregnancy and lactation, as described by Parigi Bini et al. [4, 5] and Xiccato et al. [17]. After the 21st day of pregnancy, foetal development is accelerated and maternal reserves become progressively depleted [3]. The initially exponential growth of foetuses gradually changes to linearity from day 23 [6] and the food intake of the does becomes progressively restricted due to the increasing size of the reproductive tract and the uterine contents. The usual energy content of commercial diets (10–12 MJ DE/kg) does not meet the requirement of a doe carrying more than four foetuses and results in the mobilisation of body reserves [1].

In the present study we set three main objectives: 1) to follow the changes in body composition of pregnant does using a non-invasive method; 2) to compare the X-ray CT method with the chemical analysis method; and 3) to study the influence of litter size on changes in body reserves during pregnancy.

In previous experiments changes in body composition were only studied using the comparative slaughter method; therefore the use of a non-invasive method (CT in this case) constitutes the originality of this work.

2. MATERIALS AND METHODS

2.1. Animals and experimental procedure

A total of 43 nulliparous Pannon White rabbit does were kept in individual cages (80 × 50 cm) and fed ad libitum with a commercial pelleted diet (DE 10.30 MJ/kg, crude protein 17.5 %, ether extract 3.6 %, crude fibre 12.4 %), developed for breeding does. Twenty-seven of these animals were artificially inseminated at the age of 5 months and 3854 (S.D. 198) g mean live weight. Seventeen of them became pregnant.

The feed consumption and the live weight of the does were recorded daily, but due to the 12-h fast before CT scanning only one value per week is represented in *figures 1* and *2*.

2.2. CT scanning procedures

CT scanning of pregnant does was performed on the day of insemination, on days 14, 21 and 28 of pregnancy, and a few hours after parturition. At the same time 26 non-pregnant does were also scanned as control. After a 12-h fast the does were anaesthetised with 4 mg/kg b.w. Rompun (Bayer) i.m., and fixed with belts in a stretched position in special plexiglass containers [10]. In this position movements were restricted and the legs were well separated from the rump. Three rabbits were scanned simultaneously in the same container. A total of 27 scans were taken from each animal by means of a Siemens Somatom DRG Tomograph, with 8 mm thickness and with different distances between the scans, depending on the length of the vertebral column. The scanning range extended from the scapular arch to the end of the femur, and the pictures were processed in accordance with Romvári et al. [8]. The extreme density values (e.g., air and bone) were excluded and those corresponding to fat and muscle were retained, i.e., the range from –200 to +200 of the Hounsfield scale. This range with its 400 values was reduced to 40 so-termed Hounsfield variables (HUv) by summarising the number of pixels corresponding to 10 consecutive density values on the scale. These variables were used to calculate fat and muscle indices and to plot three-dimensional histograms to demonstrate changes in body composition. The histograms were plotted by negative exponential interpolation, using SYSTAT [14] software. To

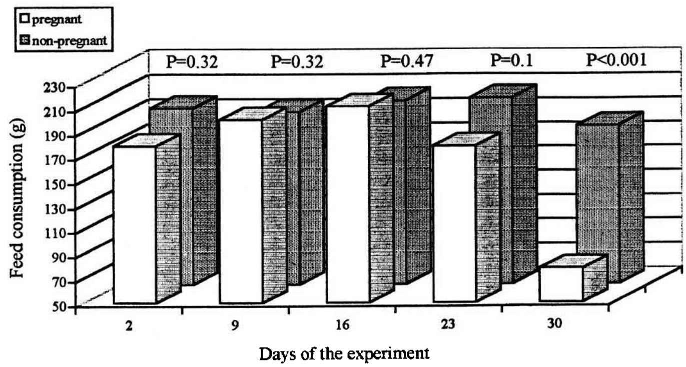

Figure 1. Feed consumption of pregnant and non-pregnant does.

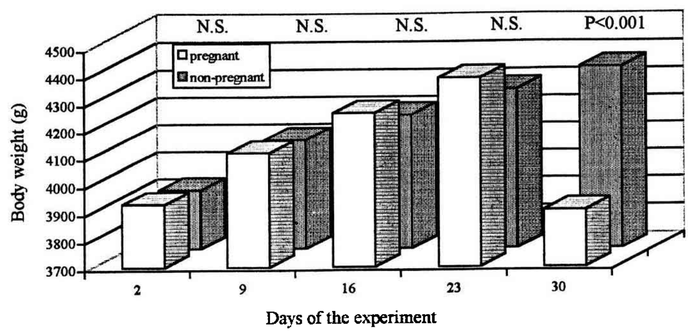

Figure 2. Body weight of pregnant and non-pregnant does.

demonstrate changes in body composition between two time points, so-termed differential histograms were plotted by subtracting the former from the latter.

2.3. Slaughter and analytical procedures

At the end of the experimental period, i.e., on the day after parturition, both experimental and control animals were slaughtered. After removal of the gut content, the rest of the body, including the empty uterus, was cut into pieces and homogenised by grinding it twice. Fat content was determined by ether extraction in accordance with the Soxhlet method.

2.4. Fat indices, muscle indices and statistical procedures

In order to compare the fat content of the does in different physiological states, Fat Indices were created from CT pictures by relating pixels corresponding to fat densities to the total number of pixels according to the formula: $(\Sigma\text{HUv } 7\text{–}16 / \Sigma\text{HUv } 1\text{–}40) \times 100$. In a previous study Romvári [7] observed that pixels corresponding to HU variables 7–16 were strongly correlated with the fat content subjected to chemical analysis, and therefore these variables were used to form fat indices. To test the predictive value of these indices, regression analysis (SPSS for Windows 7.5 [13]) was performed between the fat indices and the fat content of the body determined by a chemical method. In this way muscle indices were also created, according to the formula: $(\Sigma\text{HUv } 23\text{–}40 / \Sigma\text{HUv } 1\text{–}40) \times 100$.

Statistical analysis was carried out using analysis of variance [13], with a factorial model accounting for the fixed effect, the physiological state (pregnant or non-pregnant). The effect of litter size (small or large) was also tested. In this case only 13 of the 17 pregnant does were used, because the others had an extremely small (< 4) or an extremely large (> 9) litter size at kindling. The 13 does examined were allotted into two groups according to litter size at kindling (group 1: $n = 7$, litter size: 4–6; group 2: $n = 6$, litter size: 7–9).

3. RESULTS

3.1. Feed intake and body weight

During the first 16 days of the experimental period the daily food intake of pregnant and control does did not differ significantly (*figure 1*); however, the pregnant group had a slight tendency to increase intake with advancing time. After the acceleration of the development of foetal tissues, the feed consumption of the pregnant does lagged behind that of the control group (*figure 1*) and the difference reached the level of significance ($P < 0.05$) on the 24th day of pregnancy. From the 25th day the differences between the two groups were highly significant ($P < 0.001$).

The body weight of both pregnant and control does increased up to day 29 without significant difference. After kindling, the body weight of the experimental does returned almost to the initial level and, therefore, it differed significantly ($P < 0.001$) from that of the control (*figure 2*).

3.2. Body composition

The body composition of the rabbit does on the day of insemination is shown in *figure 3*. In the region of muscle tissue (HUv 23–40) three peaks could be observed, representing three groups of skeletal muscles (hind leg, back and foreleg muscles). In the region of fat tissue (HUv 1–18) the three smaller peaks show the fat depositions in the pelvic, perirenal and scapular regions.

Changes in body composition related to pregnancy are shown in differential histograms on *figures 4* to *7*. Little difference was seen during the first 14 days of pregnancy (*figure 4*), but a distinct peak appeared in the abdominal region between days 21 and 28 (*figure 5*).

During the last third of pregnancy loss of fat tissue could be seen in the differential histogram of *figure 6*. During the com-

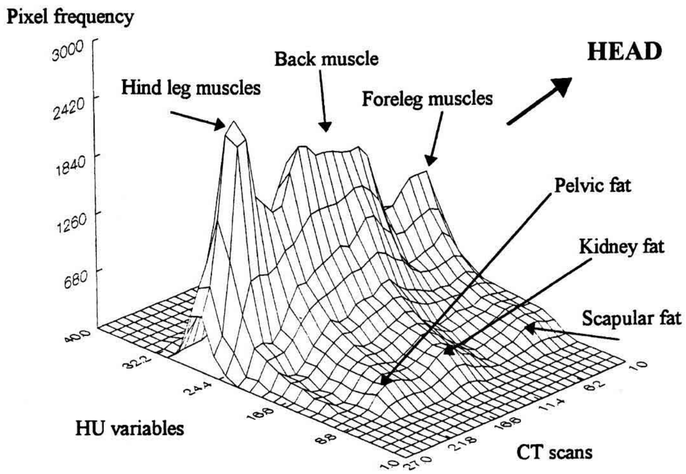

Figure 3. Body composition of the does on the day of insemination. Muscle and fat corresponds to HU variables (HUv) between 23 and 40 and between 1 and 18, respectively. The two intervals are separated by the interval of water. Extreme densities, corresponding to bone and air, are not shown.

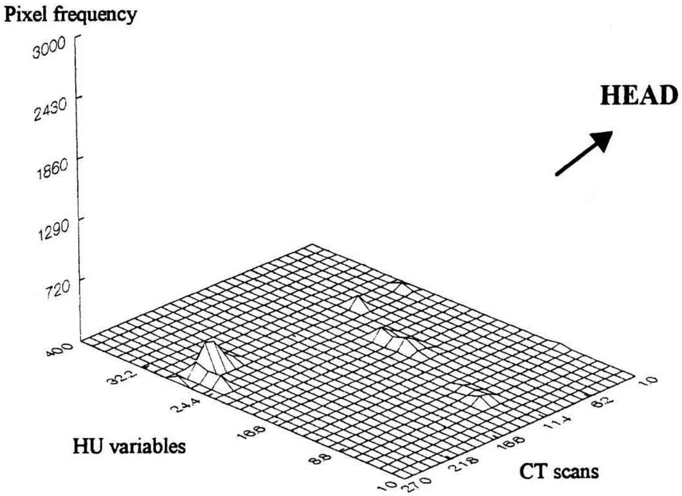

Figure 4. Changes in body composition of the does between days 1 and 14 of pregnancy, shown as a difference of two histograms.

parable experimental period the non-pregnant does (*figure 7*) accumulated fat in the pelvic, perirenal and scapular regions.

Like the histograms, the calculated fat indices also showed the growth of fat tissue in the control group during the experimental period and its decrease in the pregnant does during the last 10 days of pregnancy (*figure 8*). Inter-group differences were significant ($P < 0.001$) on days 28 and 30 of pregnancy.

To test the predictive value of estimation with these fat indices, regression analysis was carried out between the index numbers and the fat contents determined by a chemical method. The correlation was 0.93 and

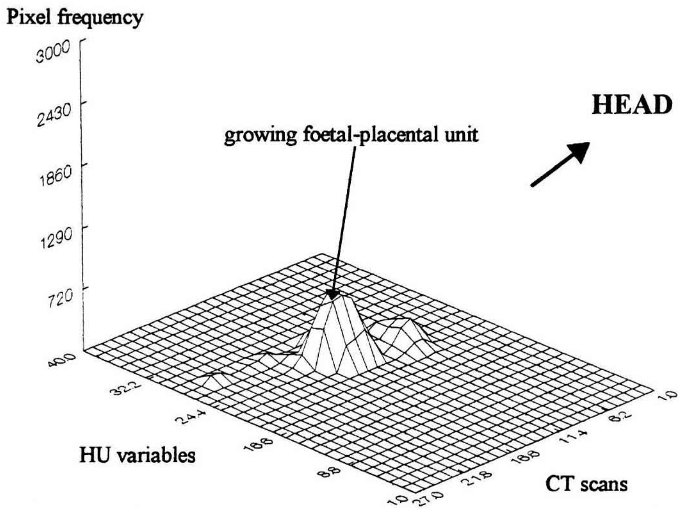

Figure 5. Changes in body composition of the does between days 21 and 28 of pregnancy, shown as a difference of two histograms.

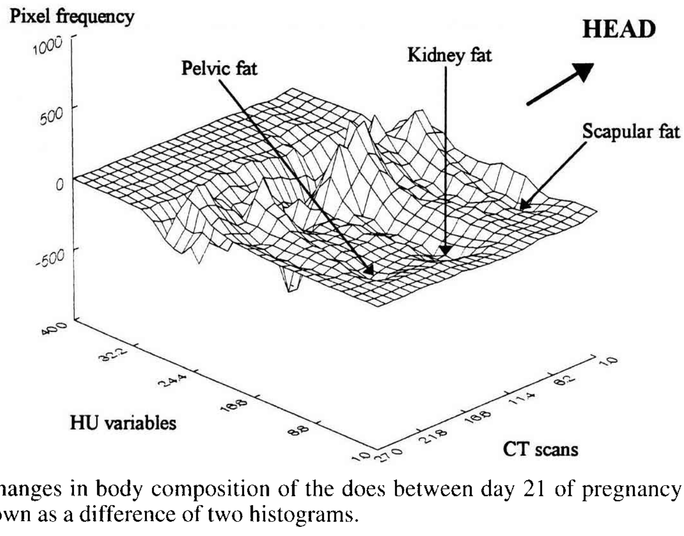

Figure 6. Changes in body composition of the does between day 21 of pregnancy and just after kindling, shown as a difference of two histograms.

0.91 for the pregnant and control does, respectively (*figures 9, 10*).

Unlike the fat indices, the muscle indices showed a decrease in muscle in the control group during the whole experimental period and in the experimental group during the first three weeks of pregnancy (*figure 11*). The ratio of pixels corresponding to muscle density values increased during the 4th week in the pregnant group and decreased again after kindling.

As was mentioned in the *Introduction*, the energy content of commercial diets usually allows only provision for four foetuses without major mobilisation of maternal reserves. It was expected, therefore, that higher litter sizes would result in higher mobilisation of body reserves. To test this

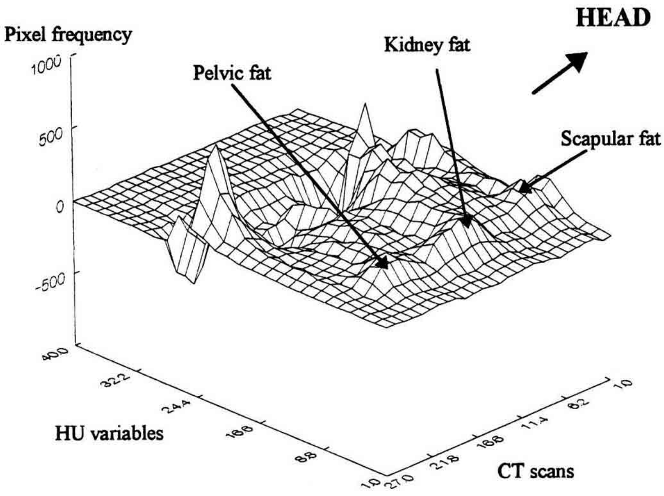

Figure 7. Changes in body composition of the non-pregnant does during the whole experimental period, shown as a difference of two histograms.

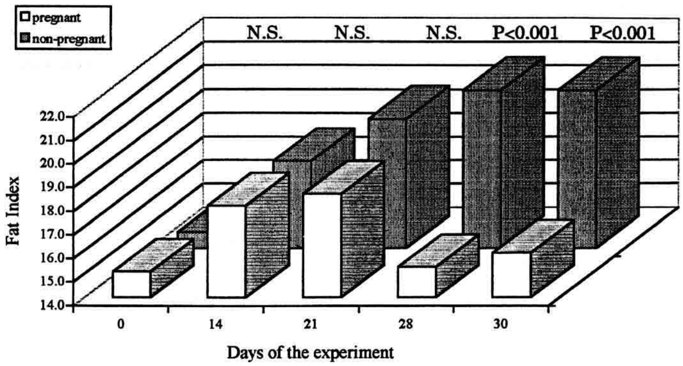

Figure 8. Changes in the fat index during the experimental period.

hypothesis, the does were allotted to two groups according to litter size, and their fat content was estimated with the fat indices (*figure 12*). In the larger (7 to 9) and smaller (4 to 6) litter size groups the fat content of the does decreased by 17.9 and 9.3 %, respectively, during the last 10 days of pregnancy ($P < 0.001$).

4. DISCUSSION

The results obtained in the present study are in agreement with previous research performed with classical slaughter techniques [2, 3, 17]. During the first 2 weeks of pregnancy, deposition of fat differed slightly between pregnant and control does, whereas during the last third an intensive mobilisation of fat took place in the maternal body to support the accelerated growth of the foetuses. A similar tendency was found by Sørensen [12], who scanned goats during pregnancy and lactation, and estimated changes in the volume of adipose tissue by CT scans, net energy balance and weight gain. Although each of the three methods had errors and assumptions, CT scanning was considered the most reliable one.

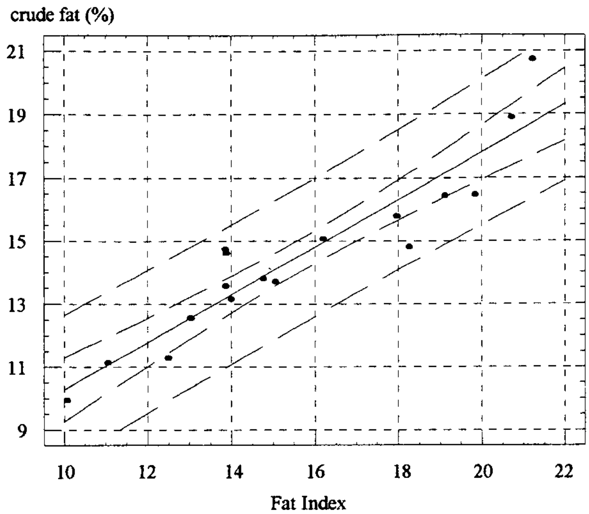

Figure 9. Correlation between fat index and crude fat content in pregnant does.

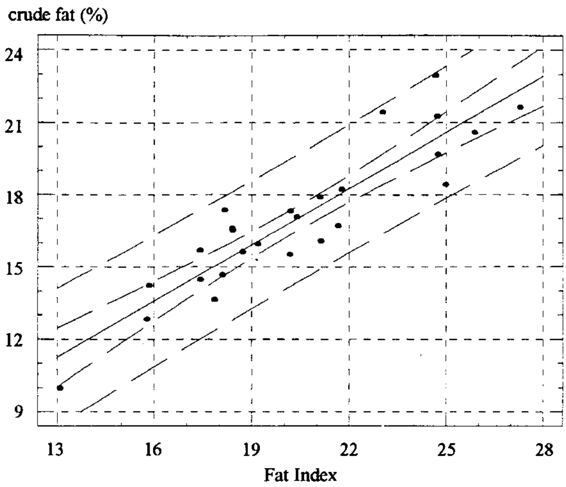

Figure 10. Correlation between fat index and crude fat content in non-pregnant does.

The decrease observed in the muscle tissue correlates to the increasing fat deposition in the control and pregnant does during the first three weeks of the experiment. The outstanding muscle index at day 28 of pregnancy is explained partly by the growing uterus and partly by the finding that the density of foetal tissues is close to that of muscle. After kindling, due to the removal of the uterine contents, the fat and muscle indices moved towards the original values.

An additional finding of the present study was the demonstration of the development of uterine, foetal and placental tissues. The peak observed in *figure 5* is located close to the line of water density (on the rim of the region considered to be muscle in the host animal), which shows, that the foetal-placental unit is rich in water and protein.

The fat indices calculated could be used to follow the changes in the fat content of the does during pregnancy. The accuracy of prediction by these indices of the fat content subjected to chemical analysis was very similar to that achieved by other authors [9, 11, 15, 16] using other prediction equations.

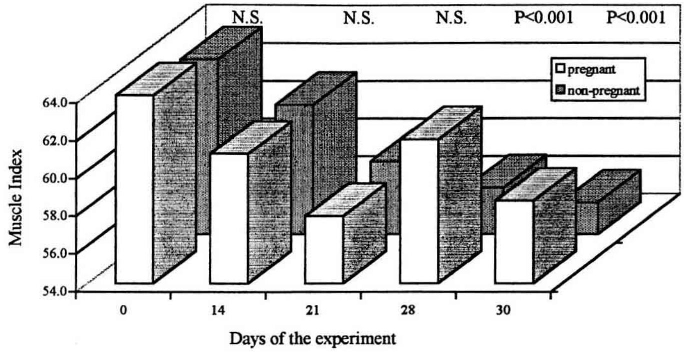

Figure 11. Changes in the muscle index during the experimental period.

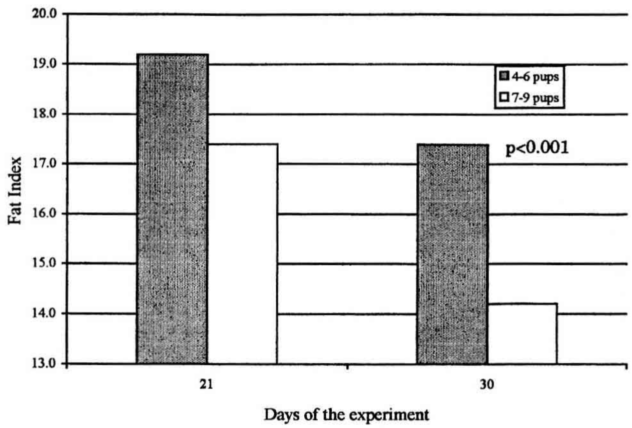

Figure 12. Changes in the fat index during the last third of pregnancy.

The difficulty in obtaining higher correlations is due to the variability of animal body composition, the limited number of test animals and the error of chemical analysis.

In the present experiment we demonstrated that CT scanning can be used to follow in a non-invasive way the changes in body composition of rabbits in different physiological states and to demonstrate the appearance of the foetal-placental unit.

ACKNOWLEDGMENT

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