World Rabbit Science

JOURNAL OF THE WORLD RABBIT SCIENCE ASSOCIATION

July - September 2018

World Rabbit Sci. 26 (3) 201 - 263

World Rabbit Science Association

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

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Journal of the World Rabbit Science Association

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Subscription Information

Volume 26, 2018, 4 issues: 80 €
For more information, contact the Editorial UPV:
pedidos@editorial.upv.es

Published by

Universitat Politècnica de València.
Camino de Vera s/n. 46071 Valencia. Spain.
Instructions to authors are available at:

www.wrs.upv.es

World Rabbit Science 2nd Series

Printed in Spain by: Byprint percom, s.l.

Legal deposit: nº V-1162-2003, Marzo 2003.

Cover design: A. Climent. Universitat Politècnica de València.

Layout: Enrique Mateo. Triskelion Disseny Editorial.

ISSN: 1257-5011

EISSN: 1989-8886

World Rabbit Sci. 2018, 26: 201-207
doi:10.4995/wrs.2018.7435

RELATIVE GROWTH IN RABBITS: THE EFFECTS OF GENETIC LINE, DIET AND GENDER

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Abstract: The relative growth of different parts of the body and tissues was analysed using an allometric model. Animals were crossbred rabbits (males and females) from the mating of commercial lines HYLA-grand parental doe (HY-GPD) female with HYLA Coloured (HY-CO) or Grimaud (GR) males, both selected for the growth rate, or HY-GPD males, selected for weaning weight. They were fed on two different diet, mainly differing in their energy content, and the relative growth of the different parts of their body was assessed. The components with a nearly isometric growth pattern, which grew at the same mean rate as the rest of the body, were skin, chilled carcass and reference carcass; hind legs for retail cuts; and dorsal and carcass length for linear measurements. All allometric coefficients were calculated with respect to the slaughter weight, except percentage of hind leg inter-intramuscular fat (IIMF), which was calculated with respect to the weight of the hind leg. The components that showed early maturing were liver, kidneys, breast and rib viscera for offal and organs; head and breast and ribs for retail cuts; and bone and IIMF percentage of the hind leg. The components with late maturing were forelegs and loin for retail cuts, inguinal, scapular and perirenal fat, meat of the hind leg, and thigh and lumbar circumference length for carcass linear measurements. The GR line showed earlier growth for loin than the maternal HY-GPD line. In addition, the GR and HY-CO lines developed scapular and inguinal fat later than the HY-GPD line. For IIMF percentage, GR presented later growth than HY-CO and HY-GPD. The main effect of the diet was on liver development and on scapular fat: rabbits fed on the high-energy diet showed later liver and scapular fat growth.

Key Words: rabbit, allometry, genetic line, feed, carcass, tissue.

INTRODUCTION

The relative growth of different parts of the body and tissues was analysed using the allometric model described by Huxley (1932). This model provides parameters with a straightforward biological interpretation that allow us to estimate the order of precocity of the different components and compare animal populations. After logarithmic transformation of Huxley's equation, a straight line is fitted to study the relative growth of the different components. However, changes in the slope of the straight line can happen during some stages of the animal's life (Cantier *et al.,* 1969; Deltoro and López, 1985). Most of the changes are concentrated in short time intervals, from 4 to 8 wk (Deltoro and López, 1985). The growth gradients postulated by Hammond (1932) are modified during postnatal life due to the existence of growth phases with different allometric coefficients (Deltoro and López, 1985).

There have been several studies regarding allometric growth comparing different genetic lines. For instance, Pascual *et al.* (2008) studied the effect of selection for growth rate on relative growth by comparing 2 contemporary groups of rabbits with the same genetic origin; one unselected group, and the other group after eleven generations of selection. Deltoro and López (1985) compared 2 lines of rabbits with different genetic origins; one selected for growth rate, and

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https://doi.org/10.4995/wrs.2018.7435

the other selected for litter size. In the present work, 2 different crossbred genetic types originated from a dam line selected for weaning weight and male lines selected for growth rate were studied; the dam line selected for weaning weight was also studied.

Regarding the effect of diet on the relative growth of the parts and tissues of the carcass, Peiretti *et al.* (2013) and Dabbou *et al.* (2017) observed that the inclusion of bilberry or tomato pomace, respectively, in rabbit diets reduced the liver percentage. Peiretti and Meineri, (2011) found a higher lipid content in the meat of rabbits fed *Spirulina platensis.* Alagón *et al.* (2015) observed that the inclusion of barley and corn dried distillers grains with solubles (DDGS) in rabbit diets led to a higher dissectible fat percentage. Pascual *et al.* (2014) pointed out that a higher fibre content in the diet of rabbits decreased the slaughter weight, dressing out percentage, meat-to-bone ratio and hind leg fat content at 63 d of age; and that switching to a high starch and fat diet at late fattening promoted liver development and fat deposition.

The aim of the current work was to analyse the relative growth of the different retail cuts and tissues and linear measurements of the carcass of the crossbreds and the lines described previously, and compare the relative growth of rabbits with different diets and of different genders.

MATERIAL AND METHODS

All experimental procedures involving animals were approved by the Universidad Politécnica de Cartagena Research Ethics Committee.

Rearing building and animals

The study was carried out at an industrial farm from November 2012 to March 2013. The farm QUIN S.A. is located at Fuente Álamo (Murcia, southeast Spain), where the climate is typically semiarid.

The experiment included a total of 2294 rabbits in a 3×2×2 factorial design, with 3 genetic crossbred types lines, 2 diets (control diet and high-energy); and 2 genders (males and females). The genetic types compared were: HYLA Grand Parental Doe (HY-GPD) selected for number born alive and weaning weight, and HYLA Coloured (HY-CO) and Grimaud PS119 (GR), both selected for average daily gain from weaning to slaughter and carcass yield at slaughter.

Since heterosis does not affect relative growth (Orengo *et al.*, 2009), 2497 purebred does (HY-GPD, France) were inseminated with semen from HY-GPD, HY-CO or GR bucks and 2440 does became pregnant. Ovulation was induced by administration of an intramuscular injection of 20 µg GnRH (Gonadoreline, Fertagyl (R), Intervet Laboratories) at the time of insemination. Litters were homogenised to 8 kits. Rabbits of the same litter stayed together with their mother until they were 7 wk old. At this time, 2294 crossbred rabbits were randomly chosen from these litters, only 1 rabbit per litter (male or female). They were moved to individual cages (45×90×40 cm^3; w×l×h) made of galvanised wire netting and kept without reproductive activity. Rabbits were weighed from 8 to 16 wk old on a weekly basis.

From weaning to slaughter, all animals were fed *ad libitum* with 2 different commercial pelleted diets (control diet=C diet and high-energy diet=HE diet). The metabolisable energy was 2100 kcal/kg for C diet (dry matter, 89%; crude protein, 15%; ether extract, 2.5%; crude fibre, 25%, starch, 8%; acid detergent fibre, 25%, neutral detergent fibre, 40%; ash, 11%) and 2400 kcal/kg for HE diet (dry matter, 89%; crude protein, 16%; ether extract, 3%; crude fibre, 17%, starch, 12%; acid detergent fibre, 22%, neutral detergent fibre, 35%; ash, 11%).

From these 2294 selected rabbits, around 254 animals from the different treatments (genetic line, diet and gender) were weighed on the farm after a fasting period of 12 hours (Slaughter weight=SW), then transported to the slaughter house, stunned by electric shock (100 V, 50 Hz) and slaughtered via exsanguination each week from age 8 to 16 weeks old, both inclusive. There were approximately 22 rabbits per genetic line, diet, gender and week of age. After exsanguination, the skin and full gastrointestinal tract were removed and weighed. Carcasses were stored at 4°C for 24 h.

Carcass dissection

At 24 h post-mortem, each chilled carcass (CC) was weighed. The liver, kidneys, thoracic viscera (the set of lungs, thymus, oesophagus, and heart, denoted as "breast and rib viscera"=BRV), and the head were removed and weighed.

The carcass obtained after removing these parts was weighed and called the reference carcass (RC) (Blasco and Ouhayoun, 1996). Several linear measurements were taken as per Blasco and Ouhayoun (1996): the dorsal length (DL) between the atlas vertebra and the 7th lumbar vertebra; the thigh length (TL) between the 7th lumbar vertebra and the distal part of Ischii; the carcass length (CL) was calculated as the sum of dorsal length and thigh length, and the lumbar circumference length (LCL) as the carcass circumference length at the level of the 7th lumbar vertebra. The perirenal, scapular and inguinal fat were separated and weighed. The dissectible fat weight was calculated as the sum of those 3 fat depots. The carcass was divided according to the dissection method used by Deltoro and López (1985), obtaining forelegs, including the insertion muscles; breast and ribs, by cutting at the joint between the last thoracic and the first lumbar vertebra; loin, including sacral vertebrae and excluding the abdominal walls; and hind legs, including the coxal bone. The right hind leg from each rabbit was weighed and dissected and meat and bone of the hind leg were weighed. The whole meat from the hind leg was vacuum packed and frozen at –20°C for further measurements. All the weights were measured in grams. All the meat of 634 hind legs was minced and the intermuscular and intramuscular fat percentage (IIMF) was determined by the indirect method near infrared spectroscopy, NIR, using the FoodScan ™ equipment.

Statistical analysis

Each variable of interest (variable y, usually the weight of the component) was related to the whole (variable x, usually slaughter weight) by Huxley's allometric equation (1932): $y=bx^k$, where b is a parameter relating the scale of measure of the whole (x) and the component (y), and k is the allometric coefficient. According to this equation, when $k<1$ the component is early maturing, when $k>1$ the component is late maturing, and when $k=1$ there is isometry, which indicates that the component and the whole mature at the same rate.

In most of the variables, y=the weight of the component and x=SW, except for IIMF percentage in which x=the hind leg weight, and for carcass linear measurements y=the cubic value of the considered variable (Pascual *et al.,* 2008).

The full model considering all data was: $\log y_{ijkl}=\beta_{ijk}+k_{ijk}\log x_{ijkl}+e_{ijkl}$, where $\log y_{ijkl}$ is the logarithm of rabbit l within group-genetic line i, diet j, sex k (logarithms in base 10) for the studied component, β_{ijk} is the value of log b for group-genetic line i, diet j, sex k, k_{ijk} is Huxley's allometric coefficient of group-genetic line i, diet j, sex k, $\log x_{ijkl}$ is the logarithm of the SW or the hind leg weight for the same individual, while e_{ijkl} is the residual.

All analyses were performed using R statistical software (2017).

It should be noted that the whole experiment was carried out with a large number of animals in order to test for interactions between effects, although no significant interaction was found. We started with the full model, which included all factors and possible interactions between the genetic line, diet and gender, and progressively models with less parameters were computed and compared using Akaike information criterion (AIC) (Blasco, 2017).

RESULTS AND DISCUSSION

Offal, organs and chilled carcass

As indicated by the allometric coefficient *k* (Table 1), components with a nearly isometric growth pattern, i.e. which grew at the same mean rate as the rest of the body, were skin, chilled carcass and reference carcass. The *k*-coefficient for the skin (Table 1) was very similar to the values reported by Pascual *et al.* (2008) for rabbits from 4 to 40 wk (1.05±0.02) or Deltoro and López (1985) for rabbits from 6 to 20 wk (1.054±0.006). Nowadays, skin is a co-product to add value in rabbit production; some markets demand it, especially the Chinese market. Our results are in line with other authors' work and confirm that skin growth is nearly isometric.

Table 1: Least square estimates (LSE) and standard errors (SE) of Huxley's allometric coefficients *k* for offal, organs, and chilled carcass with respect to slaughter weight.

Component	LSE	SE
SK	1.006	0.013
CC	1.005	0.005
RC	1.036	0.010
BRV	0.583	0.013
Lv	0.540	0.017
Ki	0.470	0.013

SK: skin, CC: chilled carcass, RC: reference carcass, BRV: breast and rib viscera, Lv: liver, Ki: kidneys.

The chilled carcass (1.005±0.005) and reference carcass (1.036±0.010) *k* coefficients were similar to the values reported by Pascual *et al.* (2008) for rabbits from 4 to 40 wk (CC=1.08±0.01; RC=1.16±0.01). The *k*-value was slightly higher for RC than for CC due to the fact that liver, kidneys, thoracic viscera and head, which are of early growth, were removed from the chilled carcass.

The components with early maturing (negative allometry) were the liver, kidneys, breast and rib viscera (lungs, oesophagus, trachea, thymus and heart), with an allometric coefficient *k* around 0.5. According to Lilja (1981) for goose, and Deltoro and López (1985) for rabbits from a line selected for growth rate and a line selected for reproductive traits, rabbits and birds are animals with high growth rates and are characterised by the early development of organs responsible for creating the energy for growth processes, especially the liver and alimentary tract. In our work, liver presented lower values (0.540±0.017) than other authors' values, such as Deltoro and López (1985) for rabbits from 1 to 20 wk, or Pascual *et al.* (2008) for rabbits from 4 to 40 wk (0.88±0.06 and 0.70±0.07, respectively). These differences could be due to differences in the ages studied. Deltoro and López (1985) found $k>1$ for rabbit from 1 to 7 wk, and from 8 to 10 the allometric coefficient was reported to be $k<1$. In the present work, a single allometric coefficient was fitted. However, some previous works in rabbit considered changes in *k* values during growth (Cantier *et al.,* 1969; Deltoro and López, 1985), and fitted more than one straight line with different values of *k* instead of a single one. Deltoro and López (1985) observed that most of these changes happened between 4 and 8 wk of age. Pascual *et al.* (2008) pointed out that Huxley's model does not provide a proper fit in components that achieve a greater weight than their mature weight in previous stages, as is the case of liver.

Regarding the effect of the genetic line on offal, organs and chilled carcass, relative growth significant differences appeared, but these differences were less than 0.1 and appeared as significant due to the small standard error of these estimates. Other authors, Butterfield *et al.* (1983) in rams, and Deltoro and López (1985) and Pascual *et al.* (2008) in rabbits did not find changes in the relative growth of these components between different genetic lines selected for the growth rate. The relative growth of these components did not differ between sexes, in agreement with the results found by Deltoro and López (1985) and Pascual *et al.* (2008) in rabbits.

The main effect of the diet was on liver development, rabbits fed on the HE diet showed slightly later liver growth than those fed the C diet (*k* difference HE–C=0.121, standard error=0.034). However, this result is not in accordance with Pascual *et al.* (2014), who observed that diets with a higher percentage of starch led to a higher percentage of liver at 63 d of age. In pigs, Weber *et al.* (2010) reported a reduction in liver weights when dietary hemicelluloses increased at the expense of starch, concomitant with lower glycogen and triglyceride liver content, suggesting that there is a repartitioning of these nutrients from the liver when high fibre diets are used.

Table 2: Least square estimates (LSE) and standard errors (SE) of Huxley's allometric coefficients *k* for retail cuts of the carcass and meat and bone of the hind leg with respect to slaughter weight.

Component	LSE	SE
H	0.717	0.008
FL	1.245	0.009
HL	1.062	0.007
BR	0.824	0.014
L	1.223	0.015
MHL	1.230	0.008
BHL	0.505	0.013

H: head, FL: forelegs, HL: hind legs, BR: breast and ribs, L: loin, MHL: muscle hind leg, BHL: bone hind leg.

Retail cuts of the carcass and meat and bone of the hind leg

Retail cuts of the carcass with early maturing were head and breast and ribs, while fore and hind legs and loin (Table 2) were found to have late maturing.

The early maturing pattern for head (*k*=0.717±0.008) was also reported by other authors (Deltoro and López, 1985; Pascual *et al.,* 2008). The pattern of breast and ribs showed early maturing (*k*=0.824±0.014) in accordance with Deltoro and López (1985), with rabbits from 1 to 8 wk (0.995±0.004), although Pascual *et al.* (2008) for rabbits from 4 to 40 wk (*k*=1.13±0.02) and Deltoro and López (1985) for rabbits from 9 to 20 wk (1.334±0.008) obtained late maturing.

Forelegs were found to present late maturing (*k*=1.245±0.009), while Deltoro and López (1985) and Pascual *et al.* (2008) obtained isometric growth.

Hind legs presented a nearly isometric pattern (k=1.062±0.007) from 8 to 16 wk, Deltoro and López (1985) found early maturing for rabbits from 6 to 20 wk (k=0.825±0.005) and late maturing from 1 to 5 wk (k=1.175±0.013).

Loin was found to have late maturing (k=1.223±0.015), as had been found by Pascual *et al.* (2008) (k=1.24±0.02) for rabbits from 4 to 40 wk and Deltoro and López (1985) for rabbits from 9 to 20 wk (k=1.050±0.017). Deltoro and López (1985) highlighted that the body parts having higher growth rates during the first phase of postnatal growth were located in the hindquarters, which, according to the pattern of locomotion characteristics of rabbits, act as a main propulsive lever. Those with higher growth rates during the second phase were in the trunk.

Our results of early growth for bone (k=0.505±0.013) are in accordance with those of Cantier *et al.* (1969) in rabbit and Whittemore (1998) and Fisher *et al.* (2003) in swine. Muscle of the right hind leg showed late growth (k=1.230±0.008), as per Deltoro and López (1985) who observed k values 1.324±0.016 from 1 to 5 wk of age and 1.208±0.003 from 6 to 20 wk of age. This is in contrast to Evans and Kempster (1979), who observed early development in swine. Vezinhet and Prud'hon (1975) reported isometry between the muscle of the hind leg and the total muscle of the carcass, as did Deltoro and López (1985) between the bone of the hind leg and the total bone of the carcass. Therefore, late growth for meat and early growth for the bone of the whole carcass can be generalised.

The effect of the genetic line, diet and gender was mainly observed for the loin. The most important difference between lines was that loin from the GR line showed earlier growth than that for the HY-GPD line (k difference GR–HY-GPD=0.165, standard error of the difference [SED]=0.037). Deltoro and López (1985) did not find differences between the rabbits selected for growth rate compared to the maternal line; and Pascual *et al.* (2008) did not observe an effect of the selection process for the growth rate after the 11th generation of selection.

In the diet effect, those rabbits fed on the HE diet showed earlier maturing in the loin than those fed on the C diet (k difference HE–C=–0.209, SED=0.029). To the best of our knowledge, no published works have studied the diet effect.

In the gender effect, the growth of the loin was earlier for females (k difference females–males=–0.141, SED=0.029). Deltoro and López (1985) and Pascual (2007) in rabbits and Rook *et al.* (1987) in pigs did not find differences between genders for retail cuts or for muscle and bone of the hind legs.

Dissectible fats of the carcass and intramuscular fat

Within the dissectible fat, inguinal, scapular and perirenal fat showed late maturity in this order, with a much higher value for perirenal fat (Table 3). For dissectible fat, our results concur with Cantier *et al.* (1969), Deltoro and López (1985) and Pascual *et al.* (2008) in rabbits; and with Fisher *et al.,* (2003) in pigs. Late development of the dissectible fat is desirable since, on the one hand, an increase of weight as fat is more expensive, and on the other hand, consumers reject fat.

The adipose deposits are of double importance for the economy of meat production. First, because of their total mass, they add to the production cost, and secondly, because of their distribution they contribute to the quality of the carcasses. Thus it is interesting to study not only the overall development of the adipose deposits during growth, but also the relative development of deposits with different locations.

Despite this fact, the partition of fat between depots and its distribution throughout the carcasses of meat animals have commanded scant attention from research workers in comparison with other aspects of growth and development. In particular, very few studies have been carried out regarding the development of inter-intramuscular fat.

Table 3: Least square estimates (LSE) and standard errors (SE) of Huxley's allometric coefficients k for different kinds of fat of the carcass (n= 2048) with respect to slaughter weight and for intermuscular plus intramuscular fat hind leg meat (n =634) with respect to hind leg weight.

Component	LSE	SE
Scapular Fa	1.669	0.089
Inguinal Fa	1.376	0.093
Perirenal Fa	2.314	0.094
DFa	1.700	0.090
IIMF	0.493	0.128

Scapular Fa: scapular fat of the carcass, Inguinal Fa: inguinal fat of the carcass, Perirenal Fa: perirenal fat of the carcass, DFa: dissectible fat of the carcass, IIMF: intermuscular + intramuscular fat percentage of the hind leg meat.

Table 4: Least square estimates (LSE) and standard errors (SE) of Huxley's allometric coefficients *k* for the carcass linear measurements with respect to slaughter weight.

Component	LSE	SE
DL	0.9710	0.0123
TL	1.3138	0.0188
LCL	1.2864	0.0166
CL	1.0655	0.0106

DL: Dorsal Length, TL: Thigh Length, LCL: Lumbar Circumference Length, CL: Carcass Length.

In our results, the IIMF percentage showed early maturing. Only one work (Vezinhet and Prud'Hon, 1975) was found that analysed the evolution of various adipose deposits in growing rabbits from 10 d to 26 wk old. In this case, the reference variable was the total dissectible fatty tissue, and they observed that perirenal fat showed late growth and subcutaneous and intermuscular fat early growth, with allometric values comprised between 0.84 and 0.94. These results can differ depending on the reference variable, Kouba and Bonneau (2009) observed that the allometric coefficient of subcutaneous and intermuscular fat decreased when the reference variable was the total fat weight in the cut in comparison with the cut weight in pigs. Vezinhet and Prud'Hon (1975) compared rabbits and lambs and pointed out that the pattern for adipose deposits development depended on the species. In lambs, perirenal and pelvic fat showed early growth and subcutaneous fat late growth, but in rabbit this pattern was inverted. Intermuscular fat was almost isometric for both species, being earlier for rabbit.

In the effect of the genetic line, rabbits from the GR and HY-CO lines developed later scapular and inguinal fat than the HY-GPD line (*k* difference HY-GPD–GR=–0.253, SED=0.100; HY-GPD–HY-CO=–0.282, SED=0.098). This result could be explained because HY-GPD is a maternal line, which will require fat deposition earlier for its reproduction function. No significant differences were observed for perirenal and the whole dissectible fat. For the IIMF percentage, rabbits from GR presented later growth than from HY-CO (*k* difference HY-CO–GR=–0.187, SED=0.083) and from HY-GPD (*k* difference HY-GPD–GR=–0.274, SED=0.087). Deltoro and López (1985) and Pascual *et al.* (2008) found no effect of selection for the growth rate on the relative growth of the dissectible fat.

The only significant effect of diet was found to be on scapular fat (*k* differences HE–C=0.275, SED=0.077), in which rabbits fed on the HE diet showed late growth. Scapular fat showed late growth and this fat deposition would require energy, although when energy is limited fat deposition is restrained in the later maturity stage of the rabbit. For inguinal, perirenal and dissectible fat the trend was the same, later growth for the HE diet, but the difference was lower and was not significant.

No gender effect was observed for fat development at these ages, in accordance with Pascual *et al.* (2008).

Carcass linear measurement

Carcass linear measurements with late maturing were TL and LCL, whereas the measurements related to the carcass length DL and CL (Table 4) were found to be nearly isometric. Deltoro and López (1985) and Pascual *et al.* (2008) also found late maturing of TL and LCL, and isometric results for DL and CL in rabbits. Conversely, Pugliese *et al.* (2003) reported early growth of body length with respect to live weight in pigs. LCL late maturing was in accordance with the loin late maturing, and the TL late maturing with the late maturing of the hind legs. In addition, LCL presented later maturing than DL, which would lead to an increase of conformation as age increased.

No significant effect of the genetic line was found on linear measurements, in agreement with Deltoro and López (1985) when comparing one line selected for growth rate with another line selected for litter size, and Pascual *et al.* (2008) when comparing 2 groups of a same line differing in eleven generations of selection for growth rate.

No effect of the diet or gender was observed in the carcass linear measurement, in accordance with Pascual *et al.* (2008).

CONCLUSION

The results of the allometric coefficients for the different components of the rabbit body are generally in accordance with other studies. As changes in the allometric coefficient can happen during growth, small differences may be due

to differences in the period studied. The genetic line only affected the relative growth of the loin and fat deposits, and the diet affected the liver and scapular fat allometric coefficients.

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World Rabbit Science

World Rabbit Sci. 2018, 26: 209-215
doi:10.4995/wrs.2018.10154

THE EFFECT OF CAROB (*CERATONIA SILIQUA*) BEAN EXTRACT ON MALE NEW ZEALAND WHITE RABBIT SEMEN

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Abstract: The carob tree (*Ceratonia siliqua*) grows naturally in the Mediterranean region. The empiric use of carob cures for their aphrodisiac properties is very common in Turkey. Thus, the experiment was conducted to determine the effects of carob bean extracts on some reproductive parameters in male New Zealand White rabbits. During the adaptation period (stage 1), 6-8 mo old rabbits were trained in semen collection for 30 d. At the beginning of the treatment period (stage 2), rabbits were assigned randomly to 2 groups of 8 animals each. For a period of 49 d (1 spermatogenesis duration), one group was treated with a daily oral dose (10 mL) of carob extract and the other group received the corresponding volume of tap water. Semen was collected weekly. Semen samples taken at week 1 and 7 were analysed separately. At the beginning of stage 2, no differences were observed in the volume and pH of the ejaculate, sperm concentration, percentage of motility, percentage of live spermatozoa, percentage of sperm plasma membrane integrity, plasma concentration of testosterone, and seminal plasma protein levels between the control and carob extract treated animals. Similarly, at the end of stage 2, there were no differences in the volume and pH of the ejaculate, motility percentage, the percentage of live spermatozoa, percentage of sperm plasma membrane integrity, and the seminal plasma protein levels between the control and the carob extract treated animals. However, sperm concentration ($P<0.05$), plasma concentration of testosterone ($P<0.05$), and percentage of change in spermatozoa concentration ($P<0.02$) between groups were affected at the end of stage 2. The data suggested that the use of carob cures prepared by boiling carob fruit could have beneficial influences on sperm concentration in rabbits.

Key Words: carob, rabbit, sperm parameters, reproduction, testosterone.

INTRODUCTION

The carob tree (*Ceratonia siliqua*) is an evergreen tree native to the Mediterranean region found in Turkey, Libya, Spain, Italy, Portugal, Morocco, and Greece, and cultivated for its edible seed pods. Several products are produced from the seedpods and these products have a promising importance in healthy and balanced feeding because of the high nutrient contents (Karkacier and Artik, 1995; Marakis, 1996).

Previous studies have shown that carob juice is rich in potassium, sodium, calcium, magnesium, iron, copper and manganese in addition to zinc, which is a nutrient vital to the healthy functioning of the male reproductive system. Furthermore, a potent antioxidant element, gallic acid, is the most abundant phenolic compound (3.27 mg/g) present in carob fruit (Ayaz *et al.,* 2007). High tannic acid (10.2 mg/dL) content of the carob could reduce blood cholesterol levels (Würsch, 1979).

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https://doi.org/10.4995/wrs.2018.10154

Carob cures are traditionally used for their aphrodisiac properties and are believed to increase sperm count in men (Saracoglu, 2011). As a result, the empiric use of carob cures prepared by boiling the carob fruit to treat low sperm count is very common. Although carob cures are commonly consumed for their aphrodisiac properties, there is no pre-planned study available in the literature on the effects of carob on the reproductive system. Thus, the purpose of the current study was to determine whether the traditional use of carob bean extract has any effects on some reproductive parameters in male New Zealand White rabbits.

MATERIALS AND METHODS

Animals and diets

This study was approved by the ethics committee of Suleyman Demirel University (08/12/2009.27.04-171531123157011-139) and care was taken to minimise the number of animals used. A total of 16 male New Zealand White rabbits (6-8 mo old) weighing 2.5 and 3.0 kg were used in the study. Rabbits were housed individually in galvanised cages (50×50×50 cm) during the experiment. The animals were kept under standard laboratory conditions (12 h dark: 12 h light and 24±4°C) during the entire experimental period.

Feed and water were provided *ad libitum*. The rabbits were fed standard commercial rabbit pellets (Ekinciler Food Company, Burdur, Turkey; 2600 kcal/kg of metabolisable energy) with 88% dry matter, 9% ash, 16% crude protein, 15% crude fibre, 1.8% mineral mixture.

Preparation of carob bean extract

Carob bean samples were collected from the same plant in Antalya, Turkey. Extracts of carob bean were prepared daily in the way used traditionally for the treatment of low sperm count. Carob beans (150 g) were cut into 2 cm pieces and boiled in 500 mL of tap water for 3 min (Saracoglu, 2011). After cooling the mixture for 20 min, carob fruits were removed from the water by centrifugation at 3000 rpm for 10 min and filtered through cheesecloth.

Experiment and sampling

Rabbits were divided randomly into 2 groups of 8 rabbits each and trained for semen collection prior to the experiment for 30 d (adaptation period). The experimental period was 49 d (spermatogenesis duration). The traditional dose of carob extract for increasing sperm count in man is 250 mL/d. Thus, rabbits in the treatment group were exposed to 10 mL of carob extract prepared daily for 7 wk. Rabbits in the control group received the same amount of tap water daily. Throughout the experimental period, semen samples were collected twice a week with 2- to 3-d intervals between each collection. The samples were obtained by means of an artificial vagina and immediately transferred to the laboratory for further processing. Semen samples at day 1 (initial) and 49 of the experiment were analysed separately. At the end of the experiment, blood samples from each rabbit were collected from the ear artery. Blood samples were centrifuged at 1457×*g* for 30 min and the serum from each sample was stored at –20°C until assayed. One day after blood sample collection, the rabbits were euthanised and wet weights of testes, epididymides and accessory sex glands (seminal vesicles, prostate and bulbourethral glands) as a whole were recorded.

Ejaculates collection and semen evaluation

All bucks were previously adapted to the semen collection procedure. Before semen collection, bucks were allowed one false mount and at the subsequent mounting, the artificial vagina was suitably positioned for penis intromission. Libido was also evaluated during semen collection based on the time of introducing the female to the male rabbits until the male rabbit starts to mount and ejaculate into the artificial vagina. Semen evaluations were performed by a single researcher in single-blinded fashion. Semen samples were immediately assessed for physical parameters of aspect, colour, volume and pH. Immediately after collection of ejaculates, the spermatozoa concentration, motility, morphology, sperm plasma membrane integrity, number of live cells and acrosomal status were determined on the raw semen.

Spermatozoa concentration

The amount of ejaculate without gel (mL) and the semen concentration (number of sperm per mL) were recorded using a graduated tube and a haemocytometer, respectively. Each ejaculate was processed to estimate the sperm concentration in duplicate by direct microscopic examination using two haemocytometer chambers. Sperm counts were made in the sperm suspension in formalin saline solution (4% formalin in 0.9% saline; a ratio of 1:100 semen:formalin), with the aid of a Thoma haemocytometer (Marienfeld GmbH & Co. KG, Lauda Königshofen, Germany) at ×400 magnification (Ata *et al.,* 2007).

Semen pH

Initial hydrogen ion concentration (pH) of semen samples was determined just after collection using a pH cooperative paper ranging from 5.5 to 9.0 with 0.5 grades (pH-Indikatorpapier Neutralit pH 5.5-9.0; Merck, Darmstadt, Germany).

Sperm motility

The percentage of motile sperm was estimated by visual examination under ×400 magnification using a phase-contrast microscope with heated stage (Ata *et al.,* 2007). Semen samples were diluted at the rate of 1:10 with isotonic phosphate-buffered saline buffer at 37.8°C. Motility estimations were performed from 3 different fields in each sample. The mean of three successive estimations was used as the final motility score.

Sperm morphology

Structural detail visualisation of sperm (major morphological abnormalities) was recorded by fixing the cells in buffered formalin saline, then viewing the unstained cells as a wet mount with phase-contrast microscopy at ×1000 magnification. At least 200 spermatozoa were evaluated for the presence, type, and incidence of each morphologic defect. Specific morphological defects, such as knobbed acrosomes, proximal protoplasmic droplets, complete separation of the acrosome and galea capitis, complete separation of the head, swollen midpieces and coiled tails were recorded (Ata *et al.,* 2007).

Sperm viability

Sperm viability was determined using an eosin-nigrosin staining mixture (EET). Spermatozoa that were not stained were classified as 'vital' and those that showed any pink or red colouration were classified as 'dead' (Ata *et al.,* 2007).

Sperm membrane integrity test (hypo-osmotic swelling test)

The percentage of sperm membrane response was evaluated using the hypo-osmotic swelling test (HOS), as previously explained by Ata *et al.* (2007). A minimum of 200 cells was observed, and spermatozoa with typically coiled tails were recorded as HOS (+).

Seminal plasma protein

Total seminal plasma protein values were measured by a refractometer (Atago, SPR-N, Japan). Semen samples (200 µL) from each collection were centrifuged at 800×*g* for 20 min. Seminal plasma was separated from the sperm cells. Ten µL of seminal plasma were placed into the refractometer for total seminal plasma protein readings.

Histopathology

After euthanasia, left testicles were placed in buffered formalin for histological examinations. All tissue samples were routinely processed into paraffin; 5-µm thick sections were stained with haematoxylin and eosin (H&E). The slides were coded and examined in a single-blind fashion by a pathologist. The minor diameter of 15 seminiferous tubules per testis measured for quantitative purposes.

Testosterone

Serum testosterone levels were measured using a rabbit testosterone ELISA kit (CSB-E06927Rb, Cusabio Biotech CO., LTD Wuhan, Hubei, China). Intra- and inter assay precision of the assay were <15%. The detection range of the kit was 0.625-10 ng/mL. Measurements were performed according to the manufacturer's instructions.

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