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Source: Folia Zoologica, 59(1) : 1-8

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: https://doi.org/10.25225/fozo.v59.i1.a2.2010

Folia Zool. – 59(1): 1 – 8 (2010)

Relation between physical condition and reproductive activity in a population of Iberian hares, *Lepus granatensis* in northern Iberian Peninsula

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Received 8 October 2007; Accepted 31 March 2009

Abstract. The aim of this study was to analyze the relation between physical condition, measured as the Kidney Fat Index (KFI), and some reproductive parameters of *Lepus granatensis* in Navarra province, Spain. Samples were collected between October 2001 and January 2003, totaling 174 hares (87 males and 87 females). All the hares were sexed and classified in three age categories (immature, young and adult). Fertile males and females were present in all monthly samples. Kidney weight was greater in females than in males for all the age classes and kidney weight variation along the year was not significant. Ranges of perirenal fat accumulated were larger in females but mean weight of fat for all hares was similar in males and females. Body weight and kidney fat weight was directly related both for males and females. Global pattern of fat deposition along the year was similar for both sexes. The amount of perirenal fat in adult fertile hares reaches maximum values just before the main reproduction period and reproductive state conditioned kidney fat levels. Pregnancy induces fat deposition in females and factors such as the number of embryos and the stage of gestation influence kidney fat levels.

Key words: kidney fat index, body condition, Lagomorpha, Navarra

Introduction

Reproduction in wild mammals is a very expensive energetic process so, in general, individuals in the best physical condition are usually the most successful in rearing offspring (Thomson & Nicoll 1986). Several methods for nutritional status and physical condition evaluation of wildlife species have been described (Kirkpatrick 1987). Kidney fat index (KFI) was proposed first by Riney (1955) as a measure of physical condition in red deer and because it is fast and very easy to calculate. It has been widely used, specially for ungulates but also for *Lepus* species (Flux 1967, 1971, Soveri & Aarnio 1983, Pepin 1987, Parkes 1989, Bonino & Bustos 1998, Farfán et al. 2004). This method is based on the assumption that the amount of perirenal fat is a reliable indicator of the total body fat and, thus, of the body's physical condition. For its calculation the perirenal fat of the left kidney is weighed and then expressed as a percentage of the weight of that kidney [*KFI = fresh weight of the perirenal fat / fresh weight of the kidney *100*]. The inclusion of kidney weight in the index allows the comparison of KFI's among animals of different body size (Bonino & Bustos 1998).

Reliability of this method has been argued because kidney weight could be affected by

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sex, age or season (Johns et al. 1984, Van Buren & Coblentz 1985). Nevertheless in *Lepus* species it has been demonstrated that this fluctuation is very small and non-significant once kidney has reached its total development (Pepin 1987, Bonino & Bustos 1998). Thus, several attempts to relate reproductive cycles and annual patterns of fat deposition in other *Lepus* species, as European hare *L. europaeus*, have been addressed (Flux 1967, Pepin 1987, Parkes 1989, Bonino & Bustos 1998). The annual reproductive cycle of this specie includes a period of sexual inactivity in autumn (Raczynski 1964, Blottner et al. 2000) and these studies demonstrate that the amount of perirenal fat varies along this yearly cycle. This variation can be explained by the balance of factors causing fat utilization (lactation in females and reproductive activity in males) and those causing deposition (pregnancy in females and testicular development in males) (Parkes 1989, Bonino & Bustos 1998). However, no specific studies concerning this matter have been published for *L. granatensis*, an endemic species of the Iberian Peninsula that, unlike *L. europaeus*, shows reproductive activity throughout the year (Alves et al. 2002, Fernández et al. 2008). A unique published work (Farfán et al. 2004) analyzes the annual pattern of perirenal fat deposition in *L. granatensis* in southern Spain, but does not relate it with reproductive parameters.

In this paper we analyze some reproductive parameters of *Lepus granatensis,* in relation with physical condition.

Study Area

This study was carried out in Navarra province (between 42º10' and 42º40' N and 1º10' and 2º20'W), northern Iberian Peninsula. The Iberian hare occupies the south of the region, covering 4 000 km^2 approximately, were climate is mostly Mediterranean with dry hot summers and mild rainy winters. The sampling area extends along the Ebro river and includes 15 hunting areas. This region is characterized by a smooth relief that ranges between 200 and 450 m in elevation. The habitat consists of a mixture of cereal crops, mainly wheat and barley, olive groves, vineyards and Mediterranean scrubland (*Thymus* sp. and *Rosmarinus* sp. mainly). Iberian hare is one of the most important game species in the study area, where it is intensively exploited.

Material and Methods

Dead animals were collected during two hunting seasons (October to January 2001/2002 and 2002/2003) directly from hunters and, additionally, an authorization was granted to shoot hares periodically during 2002. Some road-killed hares were also examined. As a total, 174 individuals (87 males and 87 females) were collected (Table 1). Because of this heterogeneous sampling, not all the individuals could be analyzed entirely, in respect of kidney and reproductive tracts. Samples were maintained in fridge and analyzed in laboratory within 15 hours after death. When this was not possible, they were frozen (-20ºC) and analyzed within 48 hours after removal from the animals. Left kidney and surrounding fat were removed and weighed on a digital balance to the nearest milligram (Riney 1955). Body weight and additional biometric parameters were also measured. For pregnant females the lean body mass was calculated, excluding the weight of embryos, that were weighed on a digital balance. Eye lenses were taken and handled as described in the literature for *Lepus* species (Suchentrunk et al. 1991). Hares were classified in three age classes; adult or young/juvenile on the basis of the presence or absence of epiphyseal distal cartilage in the radius and ulna ('Stroh's sign'; Stroh 1931) and immature when reproductive tract was clearly not developed, namely in leverets. In the very few cases where age could not be determined from the ossification stage of radius and ulna, the dry weight of the eye lenses was employed, taking into account the threshold values we have state in previous works (Fernández & Soriguer 2003).

Males were considered fertile and reproductively active when sperm was present in epididymides, regardless of being adult or young (Alves et al. 2002, Fernández et al. 2008). Immature males were not included in the analyses. For females, five reproductive stages were distinguished in the basis of mammary gland activity and presence of embryos implanted in the uterus: (i) *immature*, not completed sexual development (ii) *non-reproductive*, reproductively inactive individuals (iii) *lactating*, milk in mammary glands (iv) *pregnant*, embryos implanted in uterus and (v) *pregnant and lactating*. Females considered reproductively active were those pregnant, lactating or both pregnant and lactating.

In order to increase the significance of statistical tests, values for all parameters were pooled bimonthly because sample size was small in some months. Normality of data was tested with Kolmogrov-Smirnov and Lilliefors methods. Parametric Z and t test were used for comparison of kidney weights and also to compare perirenal fat weight between males and females and percentages of KFIs among females of different age classes. Correlation between variables as kidney weight and testis weight, body mass and kidney fat weight or accumulated weight of embryos and the KFIs was tested using parametric Pearson correlation coefficient. Analyses of variance (ANOVA) were performed to analyze the seasonal variation of kidney mass, to compare differences in KFIs values among different categories of females, mean KFIs values among pregnant females in different stages of gestation and to compare KFIs values between females with different numbers of embryos. All statistical analyses were performed using Statistica v 4.5 (StatSoft, Inc. 1995).

Table 1. Pooled bimonthly sample sizes of males and females hares of the three age classes collected for the study. Percentages of reproductive active individuals are showed for each sex.

		Males				Females			
Month	N	n (%act.)	Imm.	Juv.	Ad.	n (%act.)	Imm.	Juv.	Ad.
J-F	24	12 (91.6)	0	2	10	12 (83.3)	2	2	8
MC-AP	22	15 (73.3)	2	3	10	7 (57.1)	1	2	4
MY-JN	17	6 (50.0)	1	3	2	11 (81.9)	1	2	8
JL-A	19	9 (77.7)	0	3	6	10 (80.0)	1	2	7
S-O	17	8 (62.5)	3	0	5	9 (44.5)	3	1	5
N-D	75	37 (73.0)	9	6	22	38 (42.2)	8	11	19
Totals	174	87 (73.5)	16	15	56	87 (58.6)	16	20	51

Results

Kidney weight and KFIs

The mean kidney mass was greater in females than in males (t=4.1; p<.001) and the difference was statistically significant for the three age classes considered (p<.01) (Table 2). Kidney mass of adult males was significantly heavier than those of immatures (t=6.16; p<.001) and youngs (t=2.35; p<.05). Likewise, kidney mass of adult females was significantly heavier than those of immatures (t=6.36; p<.001) and juveniles (t=2.78; p<.05). In fertile hares, mean kidney mass variation along the year was not significant neither for males nor for females (F=1.34 and 1.24; ns respectively).

Perirenal fat and KFI

With respect to the amount of perirenal fat accumulated, mean weight in grams for all the males and females analyzed was similar (1.37 g *vs*. 1.35 g respectively; t=0.07; ns). Anyway, ranges of fat deposition were larger in females (Table 2) and both adult and young females accumulated more fat than males (Table 2). Adult females (mean KFI=35.1%) were fatter than adult males (mean KFI=31.1%), but this difference was not statistically significant (Z=1.08; ns). Young females were also fatter than males (KFIs=30.1% and 22.7% respectively), but the difference was not significant either (Z=1.3; ns). Finally, among immatures males were fatter than females (KFIs=12.1% and 6.1% respectively) but the difference was non-significant (Z=1.3; ns).

There was a significant relationship between kidney fat weight and body mass in the case of males (n=63; r=0.43; p<.01) and between kidney fat weight and lean body mass for females (n=64; r=0.43; p<.01).

Table 2. Mean weight of kidney and perirenal fat of individuals of the three age classes analyzed. KFI's values are also given.

		Kidney weight (g)		Perirenal fat weight (g)		KFI
Sex	Age	Mean ± SD	Range	Mean ± SD	Range	Mean %
Males	Imm.	4.41 ± 0.86	2.33 – 6.06	0.52 ± 0.63	0.00 – 1.97	12.1 %
	Young	5.03 ± 0.45	4.36 – 5.71	1.12 ± 0.54	0.40 – 2.04	22.7 %
	Adult	6.02 ± 1.08	4.75 – 8.94	1.84 ± 1.50	0.00 – 5.75	31.1 %
Females	Imm.	4.82 ± 0.73	3.04 – 6.34	0.30 ± 0.41	0.00 – 1.36	6.1 %
	Young	5.84 ± 0.81	4.71 – 7.86	2.14 ± 2.03	0.20 – 6.90	30.0 %
	Adult	7.04 ± 1.48	4.75 – 8.44	2.37 ± 2.71	0.00 – 7.12	35.1 %

Fat deposition pattern

Variation of KFIs of reproductive active hares along the year was similar for both sexes (Fig. 1, 2). Mean KFI decreased during winter (Dec.-Feb.) and reached lowest values in early spring (Mar.-Ap.). Thereafter KFIs increased through spring and summer (Mar.-Aug.). On males, this increase continued to a peak in early autumn (Sep.-Oct.) and decreased to lower levels in November-December. In the case of females, KFIs slightly decreased at this period, but maintained similar values until winter.

KFIs and reproduction

Males

Fertile males were recorded in all bimonthly periods, confirming continuous reproduction in *Lepus granatensis*. Moreover, adult males showed very high reproductive activity throughout the year, reaching 100% in all bimonthly periods except November-December (Fig. 1). Some young hares were also reproductively active, but the small sample size in some periods did not allow us to analyze in more detail young fat deposition pattern (Table 1). Kidney weight and testis weight were directly related (r=0.60; p<.01) showing that, in males with larger kidneys, testis development was more advanced. On the other hand, highest values in testis weight coincided with lowest KFIs values during March and April (Fig. 1). Nevertheless, for the all year, no relationship was observed between testis weight in fertile males and KFIs (r=0.07; ns).

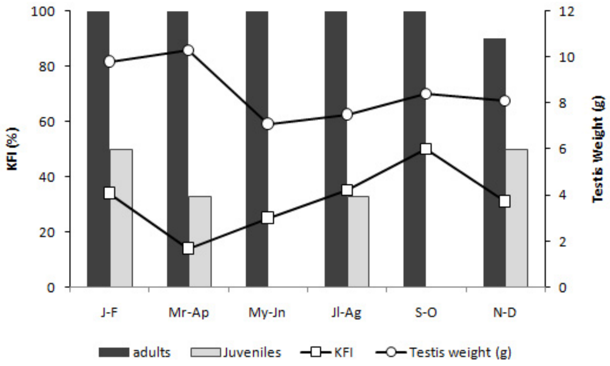

Fig. 1. Bimonthly variation in the mean kidney fat index and testis weight of reproductive active Iberian hare males in Navarra. Percentages of reproductively active adult and juvenile males are showed in bars.

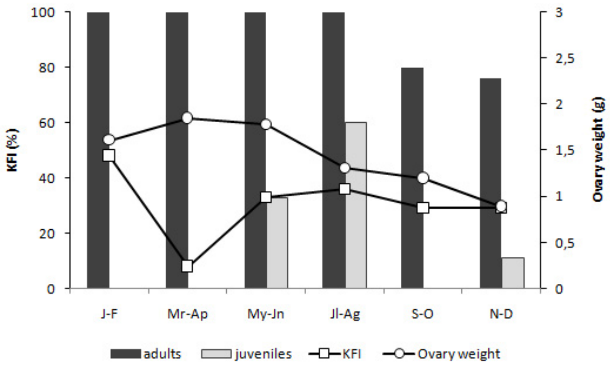

Fig. 2. Bimonthly variation in the mean kidney fat index and ovary weight of reproductive active Iberian hare females in Navarra. Percentages of reproductively active adult and juvenile females are showed in bars.

Females

With regard to females, reproductively active individuals were also present in all bimonthly periods. Just like males, percentages of adult females with any sign of reproductively activity were very high in all the periods, and only in September-October and November-December some adult females were inactive (Fig. 2). Young females were also, in some cases, reproductively active, but again sample size was very small in some periods to confirm any pattern (Table 1). The mean ovary weight of fertile females varied between bimonthly periods with the highest values observed in March- April, coinciding with the lowest kidney fat index values (Fig. 2). The relationship between mean KFIs values observed and reproductive status of females is showed in Fig. 3. Significant differences in mean KFIs values between the five categories of females could be confirmed (F=3.73; p=.007). As Fig. 3 shows, the immature individuals had the lowest values and the pregnant females the highest.

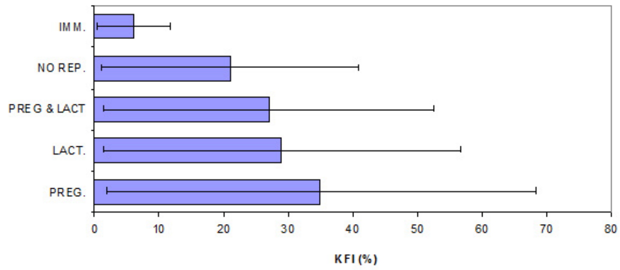

Fig. 3. Mean kidney fat indices (± 95%) of female hares in the five reproductive states considered.

KFI and gestation

Energy needed for embryos growth did not seem to affect KFIs since there was no significant relationship between KFI and the total weight of embryos for individual females (r=0.30; p=0.14, ns). Nevertheless, a tendency is observed to a higher fat deposition as embryos grow (Fig. 4). Moreover, differences in KFIs mean values among pregnant females with embryos in different stages of gestation are almost significant (F_{2df}=3.41; p=.05).

On the other hand, embryo's number seems to affect significantly the amount of accumulated fat (r=-0.38; p<.05), as females with four embryos presented the lowest values for KFI and females with single embryo the highest ones (Fig. 5). Anyway, differences in mean KFIs between females with different number of embryos were not significant (F_{3df}=2.35; p=.08; ns).

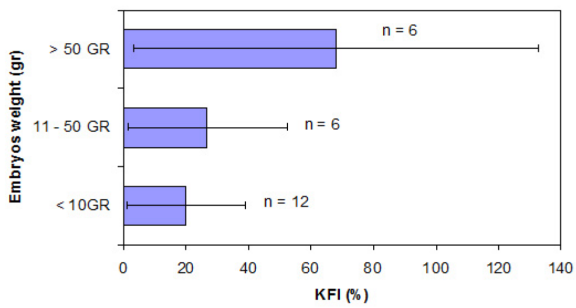

Fig. 4. Mean kidney fat indices (± 95%) of pregnant hares with embryos at different development stages. Sample size for each stage is indicated in the figure.

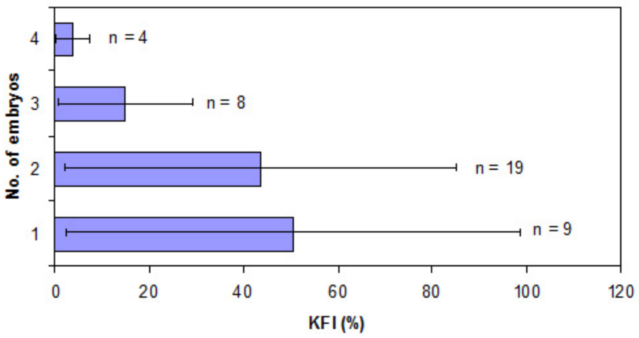

Fig. 5. Mean kidney fat indices (± 95%) of pregnant hares with different number of implanted embryos. Sample size is indicated in the figure.

Discussion

Data obtained in the present study indicate that body condition of Iberian hares is related to reproduction activity, which influences the pattern of perirenal fat deposition and utilization.

Sample size is small, mainly for juveniles, and it is quite unevenly distributed along the year, with more than 40% of samples collected during the hunting season, what could have influenced the significance of some statistical analysis. This bias against adults must be considered as a consequence of our sampling method, and not as a misinterpretation of the real age-structure of population, that is not analyzed in this work. When we collected hares, killing young individuals was avoided when possible because they provided us very little information about reproduction, the aim of our study.

Kidney weight of females was greater than that of males in all age classes. This result is in accordance with previous data showings that biometric parameters of females are significantly larger than those of males in this species (Palacios 1989, Batista & Meixa de Almeida 1996, Rodríguez & Palacios 1997). On the other hand, kidney mass of fertile males and females does not vary along the year cycle, so we considered KFIs values calculated are not affected by this variable (Flux 1971, Finger et al. 1981, Bonino & Bustos 1998).

For adult and juvenile age classes, females accumulate more perirenal fat than males, just as happens with *L. europaeus* (Bonino & Bustos 1998). On the contrary, immature males were fatter than females. Nevertheless, these differences were not significant neither in the former nor in the later case. There is no obvious reason that could explain this difference in fat accumulation trend. Maybe it reflects differences in growth rather than in nutritional status given that analyzed leverets showed quite different sizes.

Fat deposition pattern in fertile hares shows seasonal variations along the year, but the overall pattern is similar for both sexes with more clearly marked variations in the case of males. Nevertheless, these fluctuations are not easily interpreted because of the lack of a non-reproductive period along the year in this species. In *L. europaeus*, with an anoestrus period of several months during winter, it can be observed a progressive accumulation of perirenal fat starting in autumn and reaching its maximum in late winter followed by a gradual decline as breeding began (Flux 1967, Pepin 1987, Parkes 1989, Bonino & Bustos 1998). This has been interpreted as a double function of fat. First, it is used to improve survival during periods of starvation in winter (Flux 1971, Bonino & Bustos 1998) and second, it provides energy for breeding (Flux 1967, Pepin 1987, Parkes 1989, Bonino & Bustos 1998). Species not subjected to such periods of food scarcity breed normally all the year round and would not have developed this endogenous pattern of fat deposition. For example, *L. capensis* breeds continuously through the year in Africa, where food becomes extremely abundant twice a year following rainy season, and shows only a little seasonal variation in fat deposition (Flux 1969). *L. granatensis* breeds continuously too (Alves et al. 2002, Farfán et al. 2004, Fernández et al. 2008) and does not suffer a period of scarcity because its habitat diversity provides food resources throughout the year (Duarte 2000). Therefore, taking into account our results, it seems that, in males, fat accumulation is related to a progressive increase of energetic reserves towards winter, since high values of testis weights and males reproductive activity are maintained all the year round. Moreover, coincidence of highest values in testis weight and lowest KFIs values during March and April, the main reproductive period for this species in Navarra (Fernández et al. 2008), could be explained because fat reserves are employed as energetic source for spermatogenesis (Pepin 1987) and mating (Flux 1967, Parkes 1989).

For females, reproductive state of individuals conditioned kidney fat levels. Females are fatter in January and February, just before the main reproductive period in March and April recorded in Navarra (Fernández et al. 2008). Although females maintains very high levels of reproductive activity throughout the year, there is a certain degree of seasonality in this activity so that, for example, all the females analyzed in this bimonthly period were pregnant and lactating at the same time, therefore were potentially in their maximum reproductive capacity. Similar results were obtained in the south of the Iberian Peninsula, where main reproduction period occurs between February and April (Rodríguez & Palacios 1997, Alves et al. 2002, Farfán et al. 2004). Our results also suggest that gestation induces fat deposition since the three active female categories were fatter than the inactive category, while immature females hardly accumulated perirenal fat. Thus, embryos implantation induces fat accumulation (Boyd 1985, Parkes 1989), whose main function would be to provide energy for breeding (Flux 1971). Furthermore, pregnant females suffer variations in their physical condition depending on the gestation state and the number of implanted embryos, as has been observed in other *Lepus* species (Flux 1970, Boyd 1985, Angerbjörn 1986, Parkes 1989). We have observed a trend by which females with embryos in a more advanced state of development, or with lower number of embryos, reveal higher values of perirenal fat but this trend has not been confirmed statistically probably because of the small sample size. The increase of fat deposits in the last period of embryonal development could be related with a great energetic demand for *post-partum* production of milk during lactation (Parkes 1989) and that is the reason why fattest females were those only pregnant.

Finally, we agree with authors that consider KFI as a reliable indicator of body condition in hares and have used it as a body condition measure in *L. granatensis* (Farfán et al. 2004). Kidney fat is the largest and the most clear fat deposit in the body of this species, in which no other obvious fat accumulation that could be measured in both males and females has been observed.

Acknowledgements

We are very grateful to all the game rangers and hunters who contributed to the sample collection. This study was funded by the government of Navarra and an agreement between CSIC (Consejo Superior de Investigaciones Científicas) and UPNA (Universidad Pública de Navarra) made this work possible.

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