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Puppy growth rate during early periods of labrador retriever development: role of litter size and photoperiod of birth

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ABSTRACT

Several studies have recently investigated the birth weight of puppies from different pure-bred dogs. In general, birth weight decreased with litter size but there has been limited investigation into factors influencing growth during early development. The first month of life includes three stages of puppy development: *neonatal* (0–13 days), *transitional* (14–20 days), and *first week of socialisation period* (21–28 days). The aim of this study was to evaluate bodyweight and growth rate of 120 Labrador Retriever (LR) puppies during early development. Puppies from 19 show-line bitches under standardised composition of diet during pregnancy and lactation were involved in this study. Puppies were weighed weekly from birth to 28 days using an electronic digital balance. Relative weight gain (ΔW) was calculated for the neonatal period [$\Delta W_1 = (W_{day14}-W_{day0})/W_{day0}$], transition period [$\Delta W_2 = (W_{day21}-W_{day14})/W_{day14}$] and first week of socialisation period [$\Delta W_2 = (W_{day21})/W_{day21}$]. The effects of covariates on ΔW were assessed using generalised linear mixed models. Sex and photoperiod had no impact on relative weight gain, whereas litter size had a significant effect at ΔW_1 (p < .01) but not at ΔW_2 and at ΔW_3 . Relative weight gain was slightly lower in puppies from larger litter born during short photoperiod on $\Delta W1$. A deeper knowledge of the factors that significantly influence weight gain during the first four weeks of life could be useful for physiological and behavioural science as well as for canine breeding management.

HIGHLIGHTS

- 120 Labrador Retriever puppies were weighed weekly from birth to 28 days using an electronic digital balance.
- Relative weight gain (ΔW) was calculated for the neonatal period (ΔW_1), transition period (ΔW_2) and first week of socialisation period (ΔW_3).
- Litter size had a significant effect on relative weight gain at ΔW_1 but not at ΔW_2 and ΔW_3 .
- Relative weight gain was slightly lower in puppies from larger litter born during short photoperiod on ΔW_1 .

Introduction

The domestic dog (*Canis familiaris*) shows, due to the wide phenotypic variability among breeds, a wide range of body weights not found in any other species. Reliable, breed-specific estimates and ranges for normal growth are important reference data for physiological and clinical research but little information is available about factors influencing normal growth in dogs raised in typical domestic environments. Evaluation of weight requires a simple and low-cost instrument and results that are immediately available. Many studies have recently investigated the birth weight of puppies of different pure-bred dogs (Salt

et al. 2017; Groppetti et al. 2017; Schrank et al. 2019). They show that in general, birth weight decreases with larger litter size, which agrees with the finding that total litter weight is about 10–15% of the bitch weight, therefore a bigger litter is related to smaller puppies (Meyer et al. 1985). The standards of the physiological growth parameters of puppies in relation to the breed, such as weight at birth and average daily gain, can be used as a tool to monitor the daily development and to ensure neonatal survival in dogs. However, little is known about breed differences in birth weight and normal growth patterns within the early periods of puppy development (Bigliardi et al.

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ARTICLE HISTORY

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Puppy development; Litter size; Weight gain; Labrador Retriever 2013; Liotta et al. 2019; Schrank et al. 2019), a very delicate phase of life during which puppies are highly susceptible to health problems. Puppy development has traditionally been divided into four natural stages or periods: the neonatal period (from birth to 13 days), transition period (from day 14 to days 20-22), socialisation period (from 21 days to week 12) and juvenile period (week 12 to sexual maturity) (Scott and Fuller 1965). The start and end dates of these periods should be considered as flexible, especially as dog breeds vary greatly (Miklósi 2018). Weight has an impact on health and on certain behavioural aspects (Weeth 2016), with larger female puppies being more active and explorative than their smaller counterparts when subjected to a behaviour test at eight weeks of age (Wilsson and Sundgren 1998). Factors such as litter size could influence growth and potentially the start and end limits of natural development periods. In recent years, the Labrador Retriever (LR) has become the world's most popular breed (Miklósi 2018). Body weight in LR has been investigated during the neonatal period (0-7 days) (Groppetti et al. 2017; Schelling et al. 2019), first month of age (Liotta et al. 2019) from 3 to 24 months of age (Trangerud et al. 2007) and in adults (Helmink et al. 2001). However, there are few data regarding factors that influence puppy relative growth rate. Although domestic dogs are considered non-seasonal breeders, seasonal effects on the reproductive performance of bitches remain contradictory (Wei et al. 2018). Studies failed to demonstrate a season influence on litter size (Borge et al. 2011; Wigham et al. 2017), but, to the authors' knowledge, no influence on growth rate has been previously examined. The aim of the present study was, therefore, to investigate ranges of physiological body weight for LR and the effect of sex, litter size, and photoperiod of birth (long photoperiod vs short photoperiod) on body weight gain during early critical stages of puppy development.

Materials and methods

Ethical statement

The present paper is a part of a larger study designed to assess influencing factors of growth during early periods of puppy development within the framework of the programmatic initiatives of the ASPA Commission for the Breeding and Feeding of Companion Animals.

Each breeder who participated in the project signed a written agreement of cooperation. Special permission for use of animals (dogs) in this kind of study is not required in Italy. All procedures were performed in full accordance with Italian legal regulations (National Directive n. 26/14—Directive 2010/63/UE) and the guidelines for the treatments of animals in behavioural research and teaching of the Association for the Study of Animal Behaviour (ASAB). The puppies were born in kennels officially recognised by Ente Nazionale della Cinofilia Italiana (ENCI). All breeders recognised by ENCI undertake to abide by its general breeding code of ethics.

Subjects

A total of 120 healthy full-term puppies in 19 litters by 19 show-line bitches (mean body weight 31± standard deviation 2 kg), aged from 3 to 6 years were included in this study. We considered full-term gestation to be between 62 and 64 days from ovulation day and we defined breast-feeding (i.e. exclusively breastfed) as being fed only by the mother's milk; no other food or liquids were added during the examined period. From one week before parturition until the end of examined period (28 days) bitches were housed in a single, heated box (under-floor heating continuously plus a heat lamp during the first 3-5 days after whelping; temperature at puppy level was between 28 and 31 °C). According to the six breeders involved in the study, bitches were fed during pregnancy and lactation with a specific diet. The pregnant bitches were fed from the 1st day of heat to the 42nd day of gestation with the same dry feed in order to exclude the influence of bitch diet on the birth weight of pups. The diet was gradually replaced from the 43th day of pregnancy with dry puppy feed until weaning (Table 1). No pharmacological treatments were used to increase milk production (i.e. oxytocin, domperidone). The day of parturition was considered as Day 0 with all puppies weighed after colostrum intake on Day 0 and then

Table 1. Composition of the commercial diets (extruded dried
pet food) used from gestation to weaning period in Labrado
Retriever bitches.

	Gestation phase (1st–42nd day)	Starter phase (43th day–weaning)
Protein (%)	26	30
Fat (%)	18	22
Carbohydrate (%)	31.2	30.2
Crude Fibre (%)	3.1	1.9
Calcium (%)	1.1	1.28
Phosphorus (%)	0.8	1.01
Omega-3 fatty acids (%)	0.85	0.84
Omega-6 fatty acids (%)	3.12	4.01
Vitamin A (UI/kg)	39000	20500
Vitamin E (mg/kg)	600	600
Vitamin C (mg/kg)	300	410
Biotin (mg/kg)	6.88	1.3
Folic Acid (mg/kg)	29	1
Metabolisable Energy (kcal/kg)	3874	4183

weighed weekly for the first 28 days of life. Puppies were weighed using a specific digital scale (MyWeigh Ultra Baby Precision Digital Pet Scale), which is sensitive from 100 g to 20 Kg \pm 5 g. The data set initially comprised 137 pups, which were born between September 2016 and March 2018. Seventeen puppies were either stillborn or died during the first week of age and were excluded from statistical analysis (but included for the average litter calculation). Date of birth was used to consider two photoperiods, namely Short Photoperiod (SP) (=October-March) and Long Photoperiod (LP) (=April-September).

Statistical procedure

Descriptive statistics for litter size and body weight were performed using Microsoft Excel for Windows 10.

Changes in relative weight gain (ΔW) were calculated according to Alves (2020). The examined periods were the neonatal [$\Delta W_1 = (W_{day14}-W_{day0})/W_{day0}$], transition [$\Delta W_2 = (W_{day21}-W_{day14})/W_{day14}$] and first week of socialisation [$\Delta W_3 = (W_{day28}-W_{day21})/W_{day21}$]. In order to handle correlation among puppies of the same litter, the analysis was carried out using mixed models (to avoid

the pseudo-replication that using dogs of the same litter would cause). The natural logarithm transformation was applied on all the response variables to normalise the data, and the assumption of normality was checked using the Shapiro-Wilk test. The rationale of the analysis was to check whether litter size, photoperiod and sex influence the relative weight gain. So, after checking for

Table 2. General information on litters and body weight (BW) expressed in grams (g) at day 0, 7, 14, 21 and 28 in Labrador Retriever puppies from \leq 7 litter size (LS) and from >7 LS.

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	$Mean \pm SD$	Median	Q1	Q3
Litter size (3–12)	7.16 ± 2.48	7	5	9
BW at day 0				
\leq 7 LS	458 ± 75.60	450	410	492.5
>7 LS	417 ± 75.0	400	360	470
BW at day 7				
\leq 7 LS	877 ± 139	910	771	995
>7 LS	716 ± 147	700	585	810
BW at day 14				
\leq 7 LS	1346 ± 210	1360	1150	1511
>7 LS	1040 ± 212	1325	840	1250
BW at day 21				
\leq 7 LS	1977 ± 328	1980	1710	2200
>7 LS	1539 ± 367	1450	1290	1850
BW at day 28				
\leq 7 LS	2661 ± 395	2670	2415	2900
>7 LS	2282 ± 497	2180	1890	2750

Q1 means first quartile (25% percentile); Q3 means third quartile (75% percentile)



Figure 1. Growth curves with percentiles of Labrador Retriever (LR) puppies related to litter size: birth to 28 days.

any significant difference in the birth weights due to these factors, the same model was also used to analyse the relative weight gains. The linear mixed model (LMM) can be formalised as follows:

$$E (In(\Delta W)) = \beta_0 + \beta_1 sex + \beta_2 Photoperiod + \beta_3 Litter size + b_0$$

where $b_0 \sim N$ (0, Σ_B) represents the random effect due to the litter. Fixing alpha to 0.05, this was divided by the number of fitted models, holding a significance threshold equal to 0.0125. Locally weighted scatter-plot smoothing (LOESS) was used to visually analyse weight changes. All analyses were conducted in R, version 3.6.3 (R foundation for statistical computing, http://www.r-project.org). The models were fitted using the lme function in the R package nlme (Pinheiro et al. 2020).

Results and discussion

Puppies of this study were 47.5% females and 52.5% males. The largest samples were born during LP (58%). General information on litters and body weight are presented in Table 2. The mean litter size was

7.16 ± 2.48 puppies and the median was 7 puppies. Using the mean of the sample, two groups were selected: litter \leq 7 puppies and litter >7 puppies (Table 2). Figure 1 illustrates body weight weekly modification related to litter size. Tables 3–5 show the influence of sex, photoperiods, and litter size on ΔW_1 , W_2 and W_3 . The P value of litter size was significantly higher (p < .01) on ΔW_1 but not on ΔW_2 and on ΔW_3 . As showed in Figure 2, the mean of relative weight gain was different in puppies from >7 litter during ΔW_1 in relation to photoperiod of birth, with the lowest values in SP puppies. Figure 3 shows the adjusted curves generated with the model of relative weight gain related to litter size.

Analysis of the average litter size revealed a median of seven pups which is lower than the values of 8 and 7.3 reported for LR by Black et al. (2008) and Mugnier et al. (2019), higher than the value of 6 reported by Groppetti et al. (2017) but agrees with values reported by Okkens et al. (1993). As expected based on the literature, birth weight was inversely proportional to litter size, with weight reduction for each additional pup per litter (Groppetti et al. 2017). For body weight at day 0, the mean has been reported to be 412.1 g



Figure 2. Relative weight gain related to litter size and photoperiod of birth in Labrador Retriever (LR) puppies.



Figure 3. Adjusted curves generated with the model of relative weight gain during examined periods related to litter size.

Table 3. Results of a Linear Mixed Model (LMM) for the examined factors on the natural logarithm of ΔW_1 (neonatal period).

Variable	β	SE	p Value
Intercept	1.01	0.18	<.001
Sex: male	-0.01	0.04	.765
Photoperiod: long	0.004	0.17	.98
Litter Size	-0.07	0.02	<.01

The intercept represents female puppies born in the short photoperiod with litter size equal to seven. β estimated regression coefficient; SE: standard error of β ; *p*-Value: *t*-test.

Table 4. Results of a Linear Mixed Model (LMM) for the examined factors on the natural logarithm of ΔW_2 (transition period).

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Variable	β	SE	p Value
Intercept	-0.67	0.19	.001
Sex: male	0.02	0.04	.638
Photoperiod: long	-0.04	0.12	.736
Litter Size	-0.02	0.02	.541

The intercept represents female puppies born in the short photoperiod with litter size equal to seven. β : estimated regression coefficient; SE: standard error of β ; *p*-value: *t*-test.

(Groppetti et al. 2017) and 485 g (Schelling et al. 2019) and the median 405.5 g (Groppetti et al. 2017). Our observed means and the medians for LR puppies are

Table 5. Results of a Linear Mixed Model (LMM) for the examined factors on the natural logarithm of Δ W3 (first week of socialisation period).

Variable	β	SE	p Value
Intercept	-1.45	0.48	.003
Sex: male	0.05	0.04	.185
Photoperiod: long	0.64	0.35	.07
Litter Size	0.07	0.07	.311

The intercept represents female puppies born in the short photoperiod with litter size equal to seven. β : estimated regression coefficient; SE: standard error of β ; *p* Value: *t*-test.

in line with those already reported. Mean, median and quartiles of body weight at day 71,421 and 28, have not previously been reported for LR. No difference between male and female pups for birth weight and growth rate during the examined period was found. In contrast, previous studies showed the sex of the individual affected birth weight in LR (Trangerud et al. 2007; Schelling et al. 2019). A possible explanation for the apparent discrepancy between our results and those previously reported could be that results in previous studies did not consider the influence of litter size. Photoperiod is known to alter many physiological changes in seasonal mammals including food intake,

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weight gain and physical activity (Mori et al. 2014). The influence of photoperiod on weight gain, secretion of hormones involved in anabolism and milk yield have been extensively studied in livestock (Allen Tucker et al. 1984; Sarko et al. 1994; Logan et al. 2020), in wild canids (Mustonen et al. 2001) but not in domestic dogs, although a seasonal influence on growth hormone has been reported in adult dogs (Gobello et al. 2002).

Conclusions

Deep knowledge of factors affecting relative weight gain during early periods of puppy development is still lacking. Our results showed that litter size is an important factor influencing weight gain during the neonatal period but not later periods. The litter size effect should be studied more thoroughly to elucidate how maternal and environmental factors contribute to growth in dogs (Trangerud et al. 2007). Further research is needed to study puppy development related to influencing factors during early development periods.

Ethical approval statement

Special permission for use of animals (dogs) in this kind of study is not required in Italy. All procedures were performed in full accordance with Italian legal regulations (National Directive n. 26/14—Directive 2010/63/UE).

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Author contributions

L.L., M.Q. and M.G. contributed to the project administration, conceptualisation and writing the original draft; D.A and M.G. performed the analyses; L.L. and D.A. supported the acquisition and interpretation of data and contributed to writing the article. All authors gave final approval to the manuscript and any revised version submitted.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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