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Relationship between cortisol and acute phase protein concentrations in female rabbits

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ABSTRACT

Rabbit meat production in Europe is usually based on a semi-intensive system, in which lactation and gestation overlap. The demands of lactation and pregnancy are likely to be relatively stressful for female rabbits and may compromise the immune system and reproductive performance. The present study was designed to characterise circulating levels of cortisol, haptoglobin (Hp), C-reactive protein (CRP), and serum amyloid A (SAA) in non-lactating and lactating female rabbits at first and second mating, and to determine whether any relationship exists between these biomarkers and litter size.

Serum cortisol concentrations were at their greatest (mean ± SEM = 39.5 ± 3.9 nmol/L) in animals at the end of lactation. However, after weaning, cortisol concentrations were not significantly different compared to nulliparous females (19.9 ± 3.6 vs. 16.3 ± 2.2 nmol/L, respectively). The highest concentrations of circulating Hp (0.14 ± 0.01 g/L) were seen in early lactating primiparous females, and lower in nulliparous females and in rabbits after weaning. In contrast, nulliparous female rabbits showed the highest plasma CRP values (13.1 ± 1.1 mg/L). No significant differences were found for SAA. Nulliparous females had smaller litter sizes than early lactating and non-lactating primiparous female rabbits. CRP and SAA showed a positive correlation ($r = +0.24$, $P = 0.011$) and were negatively related to litter size ($r = -0.23$, $P = 0.017$ and $P = 0.032$, respectively). Cortisol and Hp were not related to CRP, SAA, nor to litter size. These results suggest a closer association between the mechanisms that regulate release of CRP and SAA, compared to those that regulate Hp production. Thus, lactation is associated with changes in several stress biomarkers. CRP and SAA might be more useful for evaluating animal welfare and for predicting subsequent reproductive performance of female rabbits.

Introduction

The most common management system in commercial rabbit farming in Europe is semi-intensive, with a minimum interval between kindling and artificial insemination or natural mating of between 10 and 12 days (Arias-Alvarez et al., 2009), resulting in overlap between lactation and gestation. Modern commercial rabbit breeds have large litter sizes of around 10 kits (Khalil and Baselga, 2002). Suckling of large litters involves a significant mobilisation of body reserves in female rabbits (Castellini et al., 2010) and an increase in social interaction between the mother and offspring during lactation (Chiang et al., 2002). Thus, lactation could be viewed as a relatively stressful period that might impact on subsequent reproductive performance.

Stress increases circulating cortisol (Möstl and Palme, 2002), which is associated with impaired immune responses to infection (Blecha and Baker, 1986; Salak et al., 1993), as well as impacting on growth and fertility (Elsasser et al., 2000; Turner et al., 2005). Measurement of cortisol concentrations in blood has been used as a marker of stress in domestic animals (Cohen et al., 1997) and more recently, acute phase proteins (APPs) have also been proposed as useful stress biomarkers (Murata et al., 2004; Eckersall and Bell, 2010). APPs are synthesised predominantly in the liver, in response to pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumour necrosis factor (TNF)-α (Heinrich et al., 1990; Yap et al., 1991). The concentration of circulating APPs can increase (positive APPs) or decrease (negative APPs) in response to injury, inflammatory or infectious processes (Cray et al., 2009). Therefore, both cortisol and APPs could be useful indicators of stress and animal welfare.

Despite the potential value of APPs as stress biomarkers, there are few published studies in rabbits that analyse the relationships between APPs and health status (Sun et al., 2005; Dishlyanova et al., 2011; El-Deeb, 2013). Our hypothesis was that lactation would be a stressful period for female rabbits and that cortisol and APP concentrations in blood would increase. The aim of the present study was to characterise plasma cortisol, haptoglobin (Hp), C-reactive protein (CRP), and serum amyloid A (SAA) in non-lactating and lactating females rabbits, i.e. at first and second mating, and to analyse

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the relationships between cortisol, APPs (Hp, CRP, and SAA) and litter size.

Materials and methods

Animals

Two groups of rabbits ($n = 27$ each group) were initially recruited from the fourth generation of lines created by means of a divergent selection process for litter size variability (Argente et al., 2010), representing females with relatively homogeneous litter sizes during their reproductive lives or those with more variable litters. Animals were housed at the Miguel Hernández University of Elche (Spain) and exposed to natural lighting, although artificial lighting was kept at 16 h light:8 h dark. Ventilation was controlled, although ambient temperature varied from season to season, ranging from 6 to 36 °C in spring/summer, and from 4 to 30 °C in autumn/winter. All animals were housed in individual cages (43 × 34 × 31 cm) until they were 18 weeks of age, then relocated into individual cages (90 × 33 × 37.5 cm). The animals were fed a commercial pelleted diet (Cunilactal, Nutreco) ad libitum.

Females were presented to a male 3 days after relocation. At second mating, females were randomly assigned into one of the following groups: females mated at 12 days after their first parturition, females mated at 24 days after their first parturition, and females mated after weaning. Litter size (LS) was recorded at first and second parities, and kits weaned at 28 days.

Sample collection and storage

All experimental procedures involving animals were approved by the Miguel Hernández University of Elche Research Ethics Committee on 21 June 2011 (Reference number DTA-MJA-001-11), according to Council Directives 98/58/EC and 2010/63/EU.

Blood samples were collected from the central ear artery into EDTA anticoagulant, centrifuged at 5000 *g* at 7 °C for 20 min and plasma stored frozen at –20 °C for up to 3 months. One blood sample was taken from each female at first and second mating. The females were non-lactating at the first blood sampling (group 1: nulliparous females, NL). At the second blood sampling, the females could have been lactating (group 2: females mated at 12 days after first parturition, i.e. early lactation in primiparous females, ELP; and group 3, females mated 24 days after first parturition, i.e. late lactation in primiparous females, LLP) or non-lactating (group 4: females mated after weaning, i.e. non-lactating primiparous females, NLP).

Analysis of plasma cortisol, Hp, CRP, and SAA

Plasma cortisol concentrations were measured using a commercially available enzyme-linked immunoassay (ELISA) kit for rodents (Endocrine Technologies) according to the manufacturer's instructions. Commercial ELISA kits for Rabbit Hp (Immunology Consultants Laboratory) and CRP (Helica Biosytems) were used and plasma SAA concentrations were determined using the Multispecies SAA ELISA kit (Tridelta Development). Two replicates were measured for each sample. The intra-assay coefficients of variation (CV) for cortisol, Hp, CRP and SAA were 4.2%, 6.8%, 8.2% and 12.5%, respectively. The inter-assay CV for cortisol, Hp, CRP and SAA were 4.1%, 6.9%, 7.4%, and 10.8%, respectively.

Statistical analysis

The Kolmogorov–Smirnov test confirmed normal distribution of data for cortisol, Hp, CRP, SAA, and LS, so these were analysed using a mixed model. The lactation status of females at mating (NL, ELP, LLP and NLP), yearly season, line (high or low litter size variability) and female hierarchical to line (random effect) were included as main effects in the model. Statistical analysis was performed using the PROC MIXED procedure of the SAS 9.2 software package (SAS Institute). The relationship between cortisol, Hp, CRP and SAA with reproductive traits of LS, was performed using principal component analysis in STATGRAPHICS (Manugistic). To assess the correlations among these traits, the PROC CORR procedure of the SAS 9.2 software package (SAS Institute) was used.

Results

Differences by lactation status, season of year, and parity

The effect of lactation status on circulating cortisol, APPs, and LS is shown in Table 1. Primiparous females at the beginning of lactation (group 2) had higher cortisol concentrations than nulliparous females (group 1), with cortisol concentrations most elevated in rabbits at the end of lactation (group 3). Non-lactating primiparous females (group 4) showed cortisol values similar to nulliparous females. The lowest Hp concentrations were seen in nulliparous females, with plasma Hp significantly elevated in all other groups, but particularly in lactating primiparous females at the beginning of lactation (group 2). Nulliparous females showed the highest plasma CRP concentrations, with lowest values seen in non-lactating primiparous females (Table 1). There were no significant differences in SAA concentrations comparing groups. Nulliparous females had lower LS than lactating primiparous females that were mated at the beginning of lactation as well as non-lactating primiparous females. However, when lactating primiparous females were mated at the end of lactation, their LS was not significantly different to those of nulliparous females (Table 1).

The season of the year had a significant effect on cortisol, Hp and CRP, but not SAA or LS (Table 1). The lowest cortisol concentrations were seen in summer ($P = 0.015$) and the highest Hp and CRP concentrations were recorded in spring ($P = 0.043$ and $P = 0.024$, respectively). There were no significant differences in plasma cortisol or Hp, comparing the two lines selected for litter size variability (Table 1). However, the line selected for increased litter size variation showed higher CRP ($P = 0.001$) and SAA ($P = 0.002$) concentrations and lower LS ($P = 0.009$) compared with the line selected for decreased litter size variation.

Table 1
Analysis of stress biomarkers in rabbits at different stages of their reproductive cycle. Least squares means with standard errors (LSM ± SE) are shown for cortisol, haptoglobin (Hp), C-reactive protein (CRP) and serum amyloid A (SAA) concentrations and the total number of kits born (litter size; LS).

	n	Cortisol (nmol/L)	Hp (g/L)	CRP (mg/L)	SAA (mg/L)	LS (*n*)
Lactation status						
Group 1: NL	54	16.3 ± 2.2^{a}	0.06 ± 0.01^{a}	13.1 ± 1.1^{a}	70.1 ± 5.4^{a}	6.8 ± 0.3^{a}
Group 2: ELP	17	22.9 ± 3.3^{b}	0.14 ± 0.01^{b}	9.4 ± 1.9^{ab}	65.1 ± 9.1^{a}	8.0 ± 0.5^{b}
Group 3: LLP	14	39.5 ± 3.9^{c}	0.12 ± 0.02^{bc}	9.9 ± 2.1^{ab}	70.5 ± 9.9^{a}	7.3 ± 0.6^{ab}
Group 4: NLP	23	19.9 ± 3.6^{ab}	0.10 ± 0.01^{c}	7.5 ± 1.9^{b}	71.1 ± 9.3^{a}	8.2 ± 0.4^{b}
P-values		<0.001	<0.001	0.012	0.511	0.097
Season of the year						
Summer	13	13.5 ± 4.4^{a}	0.09 ± 0.02^{a}	7.9 ± 2.4^{a}	69.6 ± 11.4	7.3 ± 0.5
Autumn	28	28.4 ± 3.9^{b}	0.10 ± 0.01^{a}	8.1 ± 2.1^{a}	62.9 ± 9.8	7.3 ± 0.5
Winter	36	28.7 ± 2.5^{b}	0.09 ± 0.01^{a}	10.5 ± 1.4^{a}	61.3 ± 6.5	8.0 ± 0.5
Spring	31	28.2 ± 3.3^{b}	0.14 ± 0.01^{b}	15.7 ± 1.7^{b}	72.7 ± 8.2	8.0 ± 0.4
P-values		0.015	0.043	0.024	0.696	0.749
Line (litter size variation)						
High variability	27	23.9 ± 2.2	0.11 ± 0.01	13.4 ± 1.2^{a}	75.8 ± 7.4^{a}	6.0 ± 0.3^{a}
Low variability	27	25.4 ± 2.2	0.10 ± 0.01	6.9 ± 1.3^{b}	56.1 ± 7.8^{b}	9.3 ± 0.4^{b}
P-values		0.908	0.753	0.001	0.002	0.009

Means with a different superscript letter are significantly different ($P < 0.05$). NL, nulliparous females; ELP, lactating primiparous females in the first half of their lactation; LLP, lactating primiparous females in the second half of their lactation; NLP, non-lactating primiparous females.