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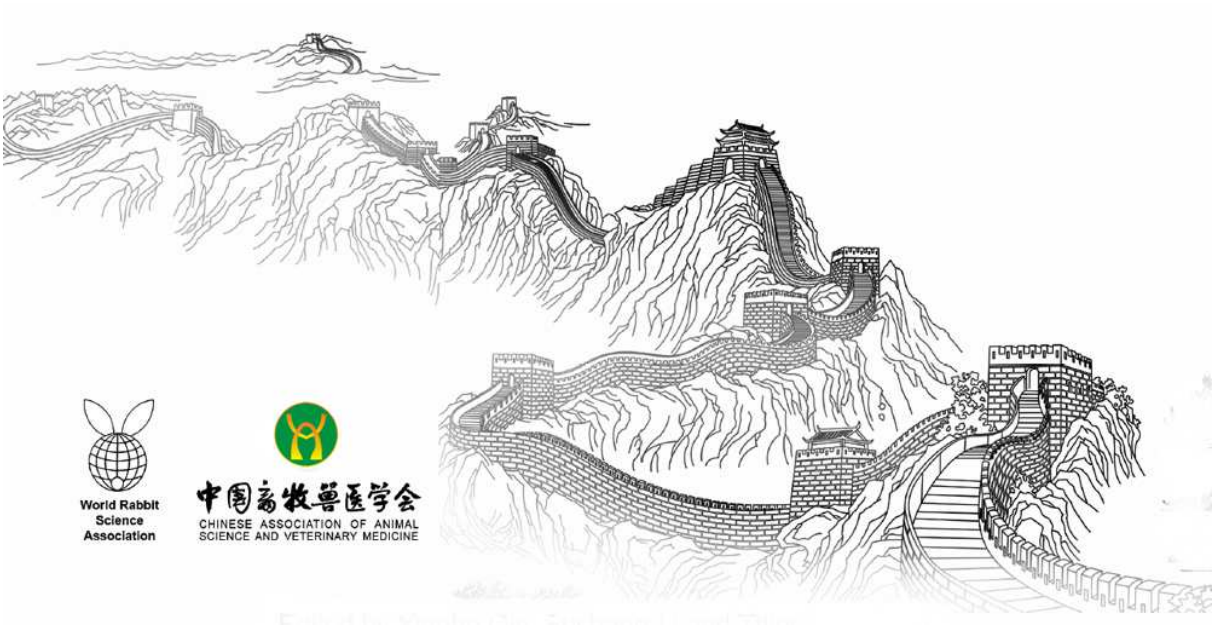

METABOLIC AND BIOCHEMICAL PRE-PARTUM CONDITIONS IN PRIMIPAROUS AND MULTIPAROUS RABBIT DOES WITH DIFFERENT LITTER SIZE

Minuti A.[1]*, Uboldi O.[2], Calamari L.[1], Piccioli-Cappelli F.[1], Bani P.[1], Ferrari A.[1], Gachiuta O.[1], Trevisi E.[1]

[1] Istituto di Zootecnica, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, via Emilia Parmense 84, 29122 Piacenza, Italy
[2] Cargill s.r.l., div CFN società del gruppo Cargill Inc., via Ripamonti 89, 20141 Milano, Italy
*Corresponding author: andrea.minuti@unicatt.it

ABSTRACT

The study investigated the pre-partum physiological and metabolic conditions in nulliparous (PR) and multiparous (MU) rabbit does and their relationship with litter size. Blood samples from 13 PR and 30 MU were collected 4 days before parturition and analysed for metabolic and inflammatory indicators and selected minerals. Body weight and number of offspring were also recorded. Data were subjected to ANOVA and stepwise regression analysis. The PR had lower body weight compared to MU (-260 g; P<0.05) and litter size (-3.0; P<0.01). However, compared to MU, PR does had a higher concentration (+0.098 mmol/L, P<0.01) of non-esterified fat acid (NEFA), and ß-hydroxybutyrate tended to be higher (+0.036 mmol/L, P=0.06) suggesting a greater body fat mobilization. PR group also showed lower concentrations of positive acute phase proteins (ceruloplasmin: -1.34 g/L and haptoglobin: -0. 5 g/L; P<0.05), total bilirubin (-0.45µmol/L, P<0.01) and globulin (-4.1 g/L) and a higher concentration of total cholesterol (+0.071 mmol/L, P<0.01), suggesting a better health status. The regression analysis of litter size on plasma parameters revealed a positive relationship for total bilirubin and aspartate aminotransferase concentrations and a negative relationship for glucose. These results indicate that last days of pregnancy are crucial for the health of doe. Moreover, important metabolic and inflammatory differences between PR and MU have been observed. PR does have a higher body fat mobilization despite the lower litter size, but a better inflammatory condition than MU. Moreover, the high number of gestated foetuses is confirmed to be a stressful factor for rabbit does. New management and nutritional strategies for reproductive rabbit does are required. Because parity order may increase the risks of suffering a metabolic condition, rearing strategies for doe should be adjusted to their age and physiological status.

Key words: rabbit does, parity order, litter size, metabolic profile.

INTRODUCTION

The improvement of reproductive management of the rabbit doe, the genetic programs and the feeding systems have greatly increased the productivity of the modern intensive rabbit breeding. At the same time, several problems related to the health status and the welfare of does have also appeared (Castellini et al., 2010; Pascual et al., 2013). Major concerns relate to the high offspring mortality, high culling rates (due to infertility or disease) and low productive performance (Rosell and de la Fuente, 2009; Sánchez et al., 2012). The genetic selection for reproductive traits have mainly focused on the improvement of litter size, leading to highly prolific lines (Baselga, 2004). In the reproductive rabbit does, a critical phase is the late pregnancy period (Xiccato, 1996; Minuti et al., 2015). In this period the doe is exposed to dramatic physiological challenges, including the abrupt change in their feed intake capacity. The last week of gestation can be very stressful for the female, especially if the number of foetuses is high (Minuti et al., 2015). The manner in which these changes occur, and how they are managed, are of great importance as they are closely linked with lactation success, the

incidence of clinical and subclinical postpartum diseases and the reproductive performance in the next gestation, all factors that affect significantly the profitability of the system (Castellini et al., 2010).

Therefore we described, in primiparous and multiparous does, their pre-partum metabolic and biochemical conditions. We also investigated the relationship of plasma parameters with the litter size.

MATERIALS AND METHODS

Animals, housing and feeding

This study complied with Italian laws on animal experimentation and ethics (DL n.116, 27/01/1992). The experiment was carried out in the experimental facilities of the Università Cattolica del Sacro Cuore, located in Northern Italy (Piacenza). The study was carried out using 13 nulliparous (**PR**) and 30 multiparous (**MU**) rabbit does from a commercial hybrid line (PS HYPLUS 19; Groupe Grimaud, Roussay, France). The PR does were 24 weeks old, while the MU does were 39 weeks old and were preparing for their third parturition. They were housed in individual flat-deck cages (0.4 × 0.6 × 0.35 m), were subjected to a constant photoperiod of 16 h of light, and does were given *ad libitum* access to water and to a commercial pelleted diet for lactating does (Purina Fertilap-6A: digestible energy = 11.79 MJ/kg of DM; crude protein = 196 g/kg of DM; ether extract = 44 g/kg of DM; crude fibre = 150 g/kg of DM).

Blood Sampling and Analysis

Four days before parturition, blood was collected from the central ear artery around 8:30 a.m. into tubes containing sodium heparin as anticoagulant (5 units per ml of blood), and body weight was measured immediately after bleeding. Blood samples were immediately cooled in ice water and centrifuged within 1-2 h at 3,500 g for 15 min at 4°C. The plasma was stored at -20°C until analysis. Samples were analysed for metabolic and inflammatory indicators and selected minerals by an automated biochemistry analyser (ILAB 650, Instrumentation Laboratory, Lexington, MA), as reported in Minuti et al., (2015). Number of offspring at calving were also recorded.

Statistical Analysis

Data were subjected to ANOVA using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The statistical model applied included the fixed effect of parity order (PR or MU) and the random effect of the doe. Moreover, the relationship between litter size and plasma parameters has been investigated by a multiple regression of litter size on the measured plasma parameters. The inclusion or not of the independent variables contributing to the explanation on the variability of litter size was evaluated by the stepwise process of the REG procedure of SAS.

RESULTS AND DISCUSSION

Four days before parturition the PR had a lighter body weight compared to MU (-260 g; $P<0.05$) and at calving, had less offspring (-3.0; $P<0.01$). The plasma parameters 4 days before parturition showed several differences considering the parity order (Table 1). From the energetic point of view, the PR does showed a higher concentration of NEFA (+0.098 mmol/L; $P<0.01$) and β-hydroxybutyrate (+0.036 mmol/L; $P<0.06$) than MU does. The positive acute phase proteins (ceruloplasmin and haptoglobin) were lower in PR does than in MU does (-1.34 µmol/L and -0.50 g/L, respectively; $P<0.05$). Other markers of acute phase response confirms the better condition of PR compared to MU. PR showed lower concentration of total bilirubin (-0.45 µmol/L, $P<0.001$), which increase indicates a possible liver distress (Assenat et al., 2004), lower concentration of globulin (-4.1 g/L; $P<0.001$) and higher concentration of total cholesterol (0.071 $P<0.001$), index of hepatic synthesis of lipoprotein. Among minerals, except the lower level in PR than MU, the plasma concentrations of minerals did not differed between PR and MU does. Alkaline phosphatase was higher in PR does (+7.2 U/L; $P<0.01$).

Table 1: Litter size at birth, body weight, and plasma parameters of nulliparous and multiparous rabbit does 4 day before parturition.

Variable	Parity order		M.S.E	P-value
	Nulliparous	Multiparous		
Female				
Number	13	30		
Live weight (kg)	4.41	4.61	0.14	0.045
Litter size at birth	8.7	11.7	9.45	0.006
Plasma metabolic and inflammatory parameters				
Glucose	7.45	7.21	0.17	0.086
NEFA (mmol/L)	0.220	0.123	0.005	<.001
β-hydroxybutirate (mmol/L)	0.124	0.088	0.004	0.060
Triglycerides (mmol/L)	0.351	0.300	0.009	0.114
Total cholesterol (mmol/L)	0.237	0.166	0.001	<.001
Urea (mmol/L)	6.10	6.65	0.94	0.099
Creatinine (µmol/L)	89.7	86.3	111.6	0.333
Ceruloplasmin (µmol/L)	4.28	5.62	3.8	0.045
Haptoglobin (g/L)	0.61	1.11	0.18	0.001
Total protein (g/L)	46.6	52.3	32.93	0.004
Albumin (g/L)	31.9	33.5	10.3	0.128
Globulin (g/L)	14.7	18.8	8.4	<.001
Paraoxonase (U/mL)	194.5	205.9	2702.2	0.510
Total bilirubin (µmol/L)	1.46	1.91	0.12	<.001
Alkaline phosphatase (U/L)	29.0	21.8	28.0	<.001
ROMs (mgH202/100mL)1	21.5	21.8	56.5	0.898
Plasma minerals				
Ca (mmol/L)	3.44	3.66	0.04	0.005
Inorganic P (mmol/L)	1.32	1.42	0.07	0.299
Mg (mmol/L)	1.14	1.23	0.03	0.137
K (mmol/L)	4.77	5.11	0.34	0.099
Cl (mmol/L)	106.5	105.3	4.9	0.099
Zn (µmol/L)	16.3	16.9	20.4	0.704

M.S.E.: Mean squared error. NEFA: non-esterified fatty acid; ROMs: reactive oxygen metabolites.

PR does showed a higher body fat mobilization (i.e. higher plasma concentration of NEFA and β-hydroxybutirate) and a lower number of gestated foetuses. Although in our study the feed intake was not measured, there is evidence that primiparous females has a lower intake capacity than multiparous ones (Pascual et al., 1998). The negative energy balance condition at the end of gestation could be worsened by the decrease of feed intake around the parturition (Xiccato, 1996), which induces hypoinsulinaemia in rabbit does (Brecchia et al., 2006), preventing the use of glucose as energy source with consequent increase of lipid catabolism and NEFA concentrations (Rebollar et al., 2011).
With the regression analysis (Table 2) we investigated how plasma parameters explains the variability observed in the number of gestated foetuses (measured as litter size at birth). We observed a positive relation between number of offspring and concentration of bilirubin and aspartate aminotransferase and negative relationships for glucose. These data support the idea that rabbit does face metabolic and inflammatory problems in the last week of gestation (Minuti et al., 2015), and these are partially related to the number of gestated fetuses.

Table 2: Regression coefficients for litter size on plasma parameters. Independent variables were selected with stepwise regression for the whole population rabbit does.

Variable	Parameter estimate	Standard error	P-values
Glucose	-2.747	0.789	0.001
Total bilirubin	4.195	0.845	<0.001
aspartate aminotransferase	0.015	0.007	0.038

CONCLUSIONS

Our study supports the evidence that the last days of pregnancy are crucial for the health of rabbit does and that there are important differences between primiparous and multiparous does for metabolic and inflammatory status. PR does have a higher body fat mobilization despite the lower litter size, but a better inflammatory status than MU. These results could be useful to design different strategies, i.e. nutritional and managerial, to meet their specific requirement. Moreover, the high number of gestated foetuses is confirmed to be a stressful factor for rabbit does health.

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