World
Rabbit
Science
Association

PROCEEDINGS OF THE 12th WORLD RABBIT CONGRESS

Nantes (France) - November 3-5, 2021

ISSN 2308-1910

This communication was accepted by the scientific committee of the Congress

but was not presented during the Congress itself,
neither face-to-face nor remotely via Internet.

EFFECT OF LITTER SIZE ON PLASMA AMINO ACIDS IN PREPARTUM RABBIT DOES

Minuti A.[12*], Uboldi O.[3], Gallo A.[1], Lopreiato V.[1], Bruschi S.[1], Piccioli-Cappelli F.[1], Ferrari A. [1], Lovotti G. [1], Gachiuta O.[1], Trevisi E.[12]

[1] Department of Animal Sciences, Food and Nutrition - DIANA, Faculty of Agriculture, Food and Environmental Science, Università Cattolica del Sacro Cuore, via Emilia Parmense 84, 29122 Piacenza, Italy
[2] PRONUTRIGEN - Centro di Ricerca sulla Nutrigenomica e Proteomica, Università Cattolica del Sacro Cuore, via Emilia Parmense 84, 29122 Piacenza, Italy
[3] Cargill Animal Nutrition, Cargill inc.
[*]Corresponding author: andrea.minuti@unicatt.it

ABSTRACT

The aim of this study was to investigate the effect of the litter size on the plasma amino acid profile in rabbit does. The blood of 30 pregnant does was sampled 4 days before parturition. The does were retrospectively grouped according to number of offspring in a high litter-size group (HI, does with > 11.6 kits; n=16) and a low litter-size group (LO, does with ≤ 11.6 kits; n=14). Data were subjected to ANOVA studies using the MIXED procedure of SAS. The HI group had lower concentrations of threonine, glycine, lysine and tryptophan and a higher level of glutamic acid ($p < 0.05$) compared with the LO group. The exclusively ketogenic amount of amino acids was lower in the HI does compared with the LO does ($p < 0.06$). These results show that a few days before delivery the plasma amino acid profile had points out that rabbit does that gave birth to a higher number of offspring showed an exacerbated catabolic condition; it was likely related to the greater energy demand needed to support the pregnancy.

Key words: periparturient rabbit doe, litter size, plasma amino acids.

INTRODUCTION

The use of modern prolific lines of rabbit does in intensive production systems leads to an increase in productivity but also causes a rise in several problems related to the does' health status (Rosell and de la Fuente 2009; Castellini et al., 2010). During late pregnancy, the does undergo dramatic physiological challenges. The last week of gestation can be very stressful, especially if the number of gestated fetuses is high (Minuti et al., 2015). Better comprehension of the factors involved in the physiology of rabbit does around the time of parturition can help to ameliorate their adaptation and welfare, as well as help to revise the nutritional requirements of the animals to match their new physiological demands (Xiccato G., 1996; Pascual et al., 2013). In our previous study, we observed does for a few days before parturition who were suffering stress from either metabolic or inflammatory disorders (Minuti et al., 2015). Interestingly, the disorders were affected by the number of offspring of the does (Minuti et al., 2015; Minuti et al., 2016). The genetic selection for reproductive traits has mainly focused on the improvement of litter sizes, leading to highly prolific lines (Pascual et al., 2013). Consequently, the increasing number of foetuses in new breeds may exacerbate the condition of the does in the last week of pregnancy. New research is needed to study the main mechanisms involved in these phenomena and develop strategies to prevent or attenuate the adverse physiological conditions.
The aim of the present study was to investigate the effect of the litter size on the plasma amino acid concentration before parturition in does with different number of gestated foetuses.

MATERIALS AND METHODS

Animals and experimental design

The study was carried out using 30 rabbit does (*Oryctolagus cuniculus*) from commercial hybrid lines (HYPLUS PS 19; Groupe Grimaud, Roussay, France). During the study period (November to December), the average daily temperatures ranged from 11.3 to 18.4 ºC and the average relative humidity ranged from 65% to 75%, as maintained by a forced ventilation system. The does were 7 months old and were preparing for their third parturition. They were housed in individual flat-deck cages (0.4 × 0.6 × 0.35 m) equipped with a drinker and a manual feeder. The does were bred under a constant photoperiod of 16 h of light per day. They were given ad libitum access to water and to a commercial pelleted diet for lactating does (Fertilap, Purina-Cargill s.r.l., Italy;) during the entire study period. The chemical composition of the diet is showed in table 1.

Table 1: Chemical composition of the commercial pelleted diet for lactating does (Fertilap spec.7A, Purina-Cargill s.r.l., Italy) administered during the study

Chemical composition	% (on a wet basis)
Dry matter	90.50
Crude protein	17.20
Fat	3.60
Crude fibre	14.80
Ash	8.17
Calcium	1.20
Phosphorus	0.73
Sodium	0.24

[1] Per kg of diet: vitamin A 18,000 IU, vitamin D3: 1,500 IU, vitamin E 75 mg, vitamin K3 1.20 mg, vitamin B1 9.7 mg, vitamin B2 8.6 mg, vitamin B6: 5.6 mg, vitamin B12 0.08 mg, niacinamide 57 mg, Ca-pantothenate: 37 mg, folic acid 5.3 mg, biotin 0.16, L-Lysine Monochloride 1,050 mg.

At –4 days from parturition, blood samples were collected around 8:30 a.m. from the central auricular artery into 2-mL tubes containing sodium heparin as an anticoagulant (5 units of heparin per mL of blood). After bleeding, the body weight was measured. Blood samples were cooled in ice water and the plasma obtained after centrifugation (3,500 × g for 15 min at 4 °C) was stored at –20 °C until analyses.

Chemical Analyses

Plasma (1 mL) was deproteinized by precipitation with picric acid (5 mL) using 0.5 mL of norleucine (2500 nM/mL) as an internal standard. Samples were centrifuged at 6,000 rpm for 15 min, and then 5 mL of supernatant was purified with a Dowex 2 x 10 resin column with 100 to 200 meshes in Cl– form. For three more times, HCl 0.02 N (5 mL) was added at the end of the process to wash the column. All materials exiting the column (sample and wash) were collected in a flask with a flat bottom and dried in a Rotavapor. Samples were then diluted with 5 mL of lithium buffer at a pH of 2.2 and filtered with a syringe filter of 0.22 micron. The amino acids were quantified using a postcolumn derivatization method that used ninhydrin as the analysis reagent and an amino acid analyzer (Jasco, Inc., Easton, MD) according to the official method (Moore and Stein, 1954). The amino acids were classified and grouped according their metabolic destination following the grouping reported in table 2.

Table 2: Grouping of amino acids according with their metabolic destination.

metabolic destination	Amino acids
Total AA	Asp, Thr, Ser, Asn, Glu, Gln, Pro, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, Lys, His, Arg, Trp
Branched-chain amino acid	Val, Ile, Leu
Essential AA	Thr, Val, Met, Ile, Leu, Phe, Lys, His, Trp
Non-essential AA	Asp, Ser, Asn, Glu, Gln, Pro, Gly, Ala, Tyr, Arg
Ketogenic AA	Thr, Ile, Leu, Tyr, Phe, Lys, Trp
Exclusively ketogenic AA	Leu, Lys
Glucogenic AA	Asp, Thr, Ser, Asn, Glu, Gln, Pro, Gly, Ala, Val, Met, Ile, Tyr, Phe, His, Arg, Trp
Exclusively glucogenic AA	Asp, Ser, Asn, Glu, Gln, Pro, Gly, Ala, Val, Met, His, Arg
Ketogenic and glucogenic AA	Thr, Ile, Tyr, Phe, Trp

Statistical Analysis

In order to evaluate the effect of litter size on blood amino acid profile, does were retrospectively divided into two groups according to the number of their offspring, either a high litter-size group (HI, does with > 11.6 kits; n=16) or a low litter-size group (LO, does with ≤ 11.6 kits; n=14). The criteria for grouping was the average litter size. The normal distribution of data was assessed by the Proc univariate of SAS (SAS Inst., Inc., Cary, NC; release 8.0) with the NORMAL statement. Data were subjected to ANOVA using the MIXED procedure of SAS. The statistical model included the fixed effect of the litter-size group. The pairwise comparison was carried out using the LSD (least significant difference) statement.

.

RESULTS AND DISCUSSION

The number of does according with the grouping by number of offspring, and the average values of main items recorded in the study are reported in Table 3.

Table 3: Number of does, offspring gestated by the does and body weight (average ±s.d.) of the does grouped according with the number of offspring as high litter size (HI) and low litter size (LO).

Items	HI	LO	All does
Number of does	16	14	30
Number of offspring	13.7±1.3	9.4±2.2	11.6±2.8
Body weight, kg	4.73±0.43	4.6±0.40	4.67±0.42

Results of the plasma amino acid profile classified according with their metabolic destination of the rabbit does at 4 days before parturition are reported in Table 4. The HI group compared to LO showed lower concentrations of threonine, glycine, lysine and tryptophan ($p < 0.05$; data not showed), and higher concentration of glutamic acid ($p < 0.05$; data not showed). For other amino acids, no significant differences were observed between groups. Based on their destination the exclusively ketogenic amino acids showed lower concentrations in the HI compared to LO does ($p < 0.06$).

Table 4: Effect of the litter size on plasma amino acid concentration (mg/100 ml of plasma) at -4 days from parturition in rabbit does grouped as high litter size (HI = 16 does) and low litter size (LO = 14 does)

	HI	**LO**	**s.e.m.**	**P<**
Total AA	69.23	71.31	2.43	0.55
Branched-chain AA	6.25	6.32	0.22	0.83
Essential AA	19.60	20.88	0.64	0.17
Non-essential AA	49.40	50.16	1.95	0.79
Ketogenic AA	13.19	14.21	0.49	0.15
Exclusively ketogenic AA	5.12	5.81	0.26	<0.06
Glucogenic AA	63.88	65.23	2.26	0.68
Exclusively glucogenic AA	55.80	56.82	2.09	0.73
Ketogenic and glucogenic AA	8.07	8.40	0.27	0.39

We investigated the effect of the litter size on the amino acid concentrations of does at 4 days before parturition. The study focused on 4 days before parturition because was previously demonstrated that that period is the most critical phase for periparturient rabbit does (Minuti et al., 2015). The current study showed interesting differences in relationship to the size of the breed of rabbits. The HI group showed lower plasma concentration for three essential amino acids (threonine, lysine and tryptophan) and one nonessential amino acid (glycine). No literature related to the amino acid profile in rabbit plasma is available to our knowledge. We hypothesized that a greater catabolic use of these amino acids to cope with the larger energy demand during the gestation of a greater number of fetuses, can cause a reduction of the concentrations of these amino acids in the plasma. Moreover, the reduction of plasma amino acids in HI does may be due to their more severe inflammatory condition (Minuti et al., 2015; Minuti et al., 2016). The plasma amino acid concentrations during inflammation are reduced due to the increased extraction of them by the liver (Zhou et al., 2016). Therefore, the lower amino acid concentrations in the HI does before parturition may be a result of the increased demand for hepatic synthesis.

CONCLUSIONS

This study demonstrated that few days before parturition the plasma amino acid concentration indicated a more severe catabolic condition in rabbit does with a high number of gestated fetuses; it is likely related to the greater energy demands needed to support the pregnancy and an early inflammatory response. New nutritional strategies should be considered to improve the health, welfare and performance of the high prolific rabbit doe and the amino acid in the plasma may be an interesting predictive marker.

ACKNOWLEDGEMENTS

This research was funded by the “Fondazione Romeo ed Enrica Invernizzi” Milan, Italy.

REFERENCES

Castellini C., Dal Bosco A., Arias-Álvarez M., Lorenzo P.L., Cardinali R., Rebollar P.G. 2010. The main factors affecting the reproductive performance of rabbit does: a review. Animal Reproduction Science 122:174–82.

Minuti A., Bani P., Piccioli-Cappelli F., Uboldi O., Bacciu N., Trevisi E. 2015. Metabolic and biochemical changes in plasma of the periparturient rabbit does with different litter size. Animal 9, 614–21.

Minuti A., Uboldi O., Calamari L., Piccioli-Cappelli F., Bani P., Ferrari A., Gachiuta O., Trevisi E., 2016 – Metabolic and biochemical pre-partum conditions in primiparous and multiparous rabbit does with different litter size. In Proceedings of the 11th World Rabbit Congress - June 15-18, 2016 - Qingdao - China, 315-318.

Moore S. Stein W.H. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. The Journal of Biological Chemistry 211, 907–13.

Pascual J.J., Savietto D., Cervera C., Baselga M. 2013. Resources allocation in reproductive rabbit does: a review of feeding and genetic strategies for suitable performance. World Rabbit Science 21, 123–144.

Rosell J.M., de la Fuente L.F. 2009. Culling and mortality in breeding rabbits. Preventive veterinary medicine 88, 120–7.

Xiccato G. 1996. Nutrition of lactating does. In Proceedings of the 6th World Rabbit Congress, 1996 Toulouse, France Vol. 1, 29–47.

Zhou Z., Loor J.J., Piccioli-Cappelli F., Librandi F., Lobley G.E., Trevisi E. 2016. Circulating amino acids in blood plasma during the peripartal period in dairy cows with different liver functionality index. Journal of Dairy Science 99, 2257–2267.