GENOTYPE AND AGE AFFECT NATIVE IMMUNITY TRAITS AND OXIDATIVE STATUS DURING SUCKLING PERIOD

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ABSTRACT

To investigate the effects of genotype and age on native immunity and oxidative status during suckling period used 40 rabbit does and 40 kits during suckling period from Native Middle-Egypt rabbits (NMER) and from New Zealand White (NZW). A total of 20 blood samples were collected and analyzed at 15 and 30 d of post-partum from rabbits does and kits for each genotype. Native immunity were included Serum Bactericidal Activity – SBA, Hemolytic complement Assay – HCA, lysozyme. Oxidative status (Reactive Oxygen Substances – ROS and total Antioxidant Capacity of plasma, TAC) were estimated in plasma. Genotype and interaction between breed and age were source of significantly variation for lysozyme. Interaction between genotype and age had significantly effect in Serum Bactericidal Activity (SBA) % and Hemolytic complement assay (HCA) CH50. SBA was increased while HCA was decreased at 30 d compared to 15 d in does of local breed (NMER). On the contrary in the does of NZW, the SBA was decreased and HCA was increased at 30 d. In rabbit kits age had a strong significantly influenced in lysozyme and HCA levels. Percentage of SBA was significantly affected by age and the interaction between breed and age. Rabbit kits were characterized by a higher HCA and lower SBA at 15 d compared to at 30 d in both of breeds. Oxidative status measurements in rabbit does inducted to the interaction between genotype and age had significantly effect in Total Antioxidant capacity (TAC) while Reactive Oxygen Substances (ROS) affected by both of breed and age. TAC values in rabbit kits were affected by breed and the interaction between breed and age. Total Antioxidant capacity (TAC) values in rabbit kits were affected by breed and the interaction between breed and age. In the present trial ROS and TAC values in does of NMER were higher than those in NZW at 30 d and this same trend observed in kits of NMER with advanced the age. This may be due to NMER rabbits as a local breed being more rustic, are used to move more and may suffer undergoing injuries when the available space is limited. Both of Lysozyme and SBA presented a significantly positive correlation with Antioxidant capacity. A positive correlation was between Lysozyme and SBA in rabbit kits that may be confirming their immune function as early defense barriers. In conclusion, in rabbit's does of local breed (NMER), blood lysozyme concentration and SBA % increased with advanced of suckling period while complement activity was decreased. An opposite trend was observed for rabbit does of NZW breed. In rabbit kits of both breed (NMER and NZW) increase values observed in serum lysozyme and SBA levels at 30 d compared to 15d post-partum during suckling period that may be indicated an effort to continuously adapt to environmental stress with advanced of age or/and to presence of specific receptors to E. coli on the intestinal epithelium with advanced suckling period in rabbit.

Key words: Native immunity, Oxidative status, Genotype, suckling period, rabbit does, rabbit kits

INTRODUCTION

The native immune system is an evolutionarily conserved, rapid defense system. A great majority of animal species rely exclusively on native immune responses while dealing with microbial insults (Kimbrell and Beutler 2001). Native immunity is primarily mediated by peptides, small proteins and pattern recognition receptors to fight off dangerous microbes (Vorbach *et al.,* 2006). The studies of

some investigators evidenced that native immunity traits are different in the variety of animal species and are influenced by breeds**.** Significant breed related differences in lysozyme concentrations and complement activity were reported in swine, turkeys, sheep, horses and cattle (Sotirov *et al.,* 2006). We hypothesize that breed composition and age are associated with differences in baseline immune status and oxidative status among different breeds of rabbit does and their kits during suckling period. Results of Dal Bosco *et al.,* 2009 showed that immune and oxidative resistance affected by genotype and it was important factor to be considered. Furthermore, it has been widely reported that the antioxidant status is associated with the health of the animal and with the specific and non-specific response of the immune system (Hildeman, 2004). We did not find enough information about baseline immune measures among rabbit breeds during suckling period in rabbit does and their kits and this motivated us to develop this study.

MATERIALS AND METHODS

Animal and housing

The trial was carried out at Ceds Breeding Station (Bani Suif Governorate), Animal Production Research Institute, Agricultural Research Center (ARC). This work continued from April to June 2010, under environmental temperature ranges: 21.7-31.0°C and 45-75% RH, respectively. Forty rabbit does and forty rabbit kits during suckling as equal number from two genotypes; Native Middle-Egypt rabbits (NMER) and New Zealand White (NZW). Does were housed in individual wire cages with standard dimensions of 50x50x35 cm and attached with nest boxes (35x 35 x35 cm) for kindling and nursing their kits. Cages were equipped with feeding hopers and nipples for automatic drink. Does were fed ad-libitum a commercial concentrate pelleted diet (17.4% crude protein, 11.3% crude fiber, and digestible energy of 10.45 MJ/kg diet).

Blood sampling

Blood samples were collected from 20 does or kits from each genotype at 15 and 30 d post-partum during suckling (n=40). Whole blood samples were collected via the marginal ear from does while via the puncture of the heart in kits by syringe. Each sample was divided into two and placed in tubes containing anticoagulant and without anticoagulant. For obtaining plasma the samples were centrifuged at 3000 rpm for 20 minutes and frozen it at -20 °C until analysis. After coagulating an anther sample blood at room temperature, the serum was extracted by centrifugation and analyzed within 24 hours.

Analytical determinations

Evaluation of the native immune traits (lysozyme, serum bactericidal activity, SBA, haemolytic complement assay, HCA) was in serum and oxidative status (total antioxidant capacity, TAC and reactive oxygen species, ROS) was in plasma. Serum lysozyme was measured by using a lyso-plate assay (Osserman and Lawlor, 1966). Percentage of SBA was performed according to Amadori *et al.* (1997) method. The HCA was carried out in microtitre plates and values were expressed as CH_{50}/50 ml (Barta and Barta, 1993). The levels of ROS are expressed as H_2O_2 (mmol/ml) and TAC (mmol/l) were determined by using commercial kits (Biodiagnostic, Egypt) according to Koracevic *et al*. (2001) and Aebi (1984), respectively.

Statistical Analyses

A linear mixed-effects model was used to analyze these variables using the mixed procedure of SAS (1999). The main fixed effects included in the model were genotype and age during suckling period and their interactions. Significance of the differences was assessed by the multiple t-tests. Residuals were tested for departures from assumptions.

RESULTS AND DISCUSSION

Genotype and interaction between breed and age were source of significantly variation for lysozyme (Table 1) and does of local breed (NMER) was lower than those in exotic breed (NZW) rabbits at different ages. Interaction between genotype and age had significantly ($P<0.01$) effect in Serum

Bactericidal Activity (SBA) % and Hemolytic complement assay (HCA) CH_{50} (Table 1). Also, HCA CH_{50} was significantly (P<0.01) influenced by age (Table 1). SBA was increased while HCA was decreased at 30 d compared to 15 d in does of NMER. On the contrary in the does of NZW, the SBA was decreased and HCA was increased at 30 d (Table 1).

Table 1. Effect of Genotype and age on native immunity and oxidative status traits in rabbit does

Variable	Age	Breed		P- Value		
		NMER	NZW	Breed	Age	Breed X Age
Lysozyme (µg/ml)	15	7.3±0.21b	11.6±0.24a	0.0242*	0.8091	0.0096**
	30	9.5±0.28ab	9.8±0.33ab			
Serum Bactericidal Activity (SBA) %	15	27.1±0.47c	31.6±0.19ab	0.6199	0.0749	0.0001**
	30	34.4±0.362a	28.7±0.42bc			
Hemolytic complement assay (HCA) CH50/50 µl	15	22.0±0.36a	22.2±0.35a	0.1319	0.0040**	0.0063**
	30	17.3±0.23b	23.6±.33a			
Total Antioxidant capacity (TAC) mmol/L	14	0.81±0.06b	1.18±0.05 ab	0.1921	0.2622	0.0010**
	30	1.58±0.02a	0.78±0.05b			
Reactive Oxygen Substances (ROS) H2O2 nmol /ml	15	0.376±0.011a	0.230±0.014b	0.0464*	0.0308*	0.0805
	30	0.393±0.009a	0.383±0.012a			

P<0.05; ** P<0.01.

In rabbit kits age had a strong significantly (P<0.01) influenced in lysozyme and Hemolytic complement assay (HCA) levels (Table 2). Percentage of Serum Bactericidal Activity (SBA) was significantly affected by age and the interaction between breed and age (Table 2). In details, rabbit kits were characterized by a higher HCA and lower SBA at 15 d compared to at 30 d in both of breeds (Table 2). Lysozyme values observed in rabbit does and their kits were lower than those reported by Dal Bosco et al 2009 (range from 20.64 to 12.53 µg/mL) while, it closely to observation of Bonnafous and Raynaud (1980) lysozyme values of 10.7 µg/mL in rabbits. Lysozyme was first discovered by Fleming (1922) in the nasal mucus. Lysozyme is a hydrolytic enzyme has been recognized to possess many physiological and functional properties and it have a high microbicidal activity remains (Benkerroum 2008). Also it has a synergic action with immune humoral response, and factors of the serum complement. So, Lysozyme titration is essentially related to the function of the macrophage system and basically indicates the presence of inflammation Carroll and Martinez (1979).

Table (2): Effect of Genotype and Age on native immunity and oxidative status traits in rabbit kits

Variable	Age	Breed		P- Value		
		NMER	NZW	Breed	Age	Breed X Age
Lysozyme (µg/ml)	15	8.0±0.27b	7.7±0.24b	0.1577	0.0051**	0.2657
	30	11.3±0.24a	9.2±0.24ab			
Serum Bactericidal Activity (SBA) %	15	21.0±3.0c	26.2±6.7bc	0.5546	0.0427*	0.0008**
	30	32.1±7.2a	29.2±6.3ab			
Hemolytic complement assay (HCA) CH50/50 µl	15	22.6±0.48a	20.2±0.59ab	0.2349	0.0005**	0.8324
	30	15.7±0.43bc	14.0±0.60c			
Total Antioxidant capacity (TAC) mmol/L	15	1.50±0.02a	0.77±0.03b	0.0233*	0.0839	0.0180*
	30	1.45±0.09a	1.46±0.03a			
Reactive Oxygen Substances (ROS) H2O2 nmol /ml	15	0.40±0.009	0.38±0.013	0.6212	0.0640	0.9342
	30	0.33±0.011	0.31±0.011			

P<0.05; ** P<0.01.

The value of SBA % and HCA in rabbit does and their kits (Table 1 and 2) were within values of Moscati *et al.,* 2008 which ranged 9.0 to 90.7 % and 0.16 to182.40 CH_{50}, respectively. SBA is a major parameter of native immunity and it have bactericidal activity of mammalian serum as nonspecific host defense mechanisms and may play an important role in the initial stages of microbial attack Moscati et al 2008. The increase values observed in serum lysozyme and SBA levels at 30 d compared to 15d in kits (Table 2) may be indicated an effort to continuously adapt to environmental stress (Mugnai *et al*., 2008) with advanced of

age or/and to presence of specific receptors to E. coli on the intestinal epithelium with advanced suckling period in rabbit (Gallois *et al.*, 2007) that stimulated to substrate native immunity. Also, Ponti *et al.* (1989) and Sensi *et al.* (2006) showed an age-related effect in the serum lysozyme of calves and pig. The complement system was first discovered in the serum by Jules Bordet in 1894 as a heat-labile factor that facilitated the killing of bacteria by specific antibodies (Ogundele, 2001). The complement system plays an important role in the host defence mechanisms, such as in immune bacteriolysis, immuneadherence, immunoconglutination and in enhancement of phagocytosis (Barret, 1983). The complement system is one of the earliest systems to be fully established in mucosal tissues during the neonatal period (Chernyshov and Slukin, 1989). The serum complement system consists of at least 19 proteins, mostly in pre-activated enzymatic forms (Ogundele, 1999).

Oxidative status measurements in rabbit does inducted to the interaction between genotype and age had significantly (P<0.01) effect in Total Antioxidant capacity (TAC) while Reactive Oxygen Substances (ROS) affected by both of breed and age (Table1). TAC values in rabbit kits were affected by breed and the interaction between breed and age (Table 2). Genotype and age had not significantly influenced in ROS as shown in Table 2. Oxidative stress, resulting from an increased production of free-radicals and ROS, and/or a decrease in antioxidant capacity, damages biological macromolecules and disrupts normal metabolism and physiology (Tse *et al.*, 2004). In the present trial ROS and TAC values in local breed (NMER) were higher than those in NZW at 30 d and this may be due to NMER rabbits as a local breed being more rustic, are used to move more and may suffer undergoing injuries when the available space is limited. Conform that Dal Bosco, 2009 when reported that LP rabbits (rustic genotypes) being more susceptible to oxidative stress in cage than in pen. Moreover, genotype LP reared in cage was more susceptible to oxidative stress than genotype H reared under the same conditions. Also, this same trend observed in kits of NMER with advanced the age (Table 2).

Table 3: Correlation coefficient between native immune and oxidative status traits in rabbits does

	Lysozyme	Hemolytic Complement Assay	Serum Bactericidal Activity	Antioxidant capacity
Hemolytic Complement Assay	0.002			
Serum Bactericidal Activity	0.017	-0.29		
Antioxidant capacity	0.11*	-0.32*	0.41**	
Reactive Oxygen Substances	-0.29	-0.042	-0.19	0.035

P<0.05; ** P<0.01.

Both of Lysozyme and SBA presented a significantly positive correlation with Antioxidant capacity (Table 3). These were harmony with results of Moscati *et al.*, 2008. Lysozyme and HCA were very low correlated (Table 3). There was a positive correlation between lysozyme and TAC and this is probably due to the presence of sub-inflammatory processes which enhance the release of lysozyme by neutrophiles and macrophages, and reduce the free complement which is mainly found in immuno-complexes (Moscati *et al.*, 2008.). The same cause may be explanted the negative relationship between lysozyme and ROS (Table 3).

Table 4: Correlation coefficient between native immune and oxidative status traits in rabbits kits

	Lysozyme	Hemolytic Complement Assay	Serum Bactericidal Activity	Antioxidant capacity
Hemolytic Complement Assay	0.041			
Serum Bactericidal Activity	0.11	-0.32*		
Antioxidant capacity	0.07	-0.12	0.09	
Reactive Oxygen Substances	-0.08	0.20	-0.21	0.10

* P<0.05

A positive correlation between Lysozyme and SBA in rabbit kits (Table 4) may be confirming their immune function as early defense barriers as reported by Moscati *et al.*, 2008. HCA and SBA were negatively correlated (P<0.05) and this agree with results of this Moscati *et al.*, 2008. Previous study

for Dal Bosco *et al*., 2002 indicated to there was a positive correlation between the ROS values and the antioxidant response of the animal as shown in Table 4.

CONCLUSIONS

In rabbits do of local breed (NMER), blood lysozyme concentration and Serum Bactericidal Activity (SBA) % increased with advanced of suckling period while complement activity was decreased. An opposite trend was observed in rabbit does of NZW breed. In rabbit kits of both breed (NMER and NZW) increase values observed in serum lysozyme and SBA levels at 30 d compared to 15d post-partum during suckling period and this may be indicated an effort to continuously adapt to environmental stress with advanced of age or/and to presence of specific receptors to E. coli on the intestinal epithelium with advanced suckling period in rabbit. To better understanding the dynamics and interaction between such traits during suckling period are needed to more studies especially later of suckling.

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REFERENCES

Aebi H. 1984. Catalase in vitro. *In Methods Enzymol., 105, Academic Press, 121-126.*

Amadori, M., Archetti, I.L., Frassinelli, M., Bagni, M., Olzi, E., Caronna, G., Lanterni, M., 1997. An immunological approach to the evaluation of welfare in Holstein Frisian cattle. *Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia, Brescia.*

Barta, V., Barta, O. 1993. Testing of Hemolitic Complement and its components. *Vet. Cl. Imm. Lab., Bar-Lab, Blacksburg, USA.*

Carroll S.F., Martinez R.J. 979. Role of rabbit lysozyme in in-vitro serum and plasma serum bactericidal reactions against Bacillus subtilis. *Infect. Immun., 25: 810-819.*

Dal Bosco A., Mugnai C. , Mourvaki E., Cardinali R, Moscati L, Paci G, Castellini C.2009. Effect of genotype and rearing system on the native immunity and oxidative status of growing rabbits. *Italian jounal of animal science. Vol 8, No 2s, 781-783*

Dal Bosco, A., Castellini, C., Mugnai, C. 2002. Rearing rabbits on wire net floor or straw litter: behaviour, growth and meat qualitative traits. *Livest. Prod. Sci., 75, 149-156.*

Hildeman D.A. 2004. Regulation of T-cell apoptosis by reactive oxygen species. Free Rad. Biol. Med., 36: 1496-1504.

Koracevic D., Koracevic G., Djordjevic V., Andrejevic S., Cosic V. 2001. Method for the measurement of antioxidant activity in human fluids. *J. Clin. Pathol., 54, 356-361.*

Moscati L., Dal Bosco A., Battistacci L., Cardinali R., Mugnai C., Castellini C. 2008. Native immunity and oxidative traits of growing rabbits. *World Rabbit Sci., 16: 213 - 220*

Mugnai, C., Dal Bosco, A., Moscati, L., Battistacci, L., Mourvaki, E., Cardinali, R., Castellini, C. 2008. Pasture availability and genotype effect in rabbit. *Proc. 9th World Rabbit Congress, Verona.*

Osserman, E.F., Lawlor, D.P., 1966. Serum and urinary lysozyme (muramidase) in monocytic and monomyelocitic leukemia. *J. Exp. Med., 124, 921-952.*

SAS 1999. SAS user's guide: Statistical Analysis System Institute, *SAS Inst. Inc., Cary NC, USA.*

Vorbach C., Capecchi M. R., and Penninger J. M. 2006 .Evolution of the mammary gland from the innate immune system? *Bio Essays 28:606–616.*

Kimbrell DA, Beutler B. 2001. The evolution and genetics of innate immunity. *Nat Rev Genet 2:256–267.*

Sotirov L., Semerdjiev V., Maslev T., Gerchev G. 2006. Breed and age-related differences in lysozyme concentrations and complement activity in rams. *Trakia Journal of Sciences, Vol. 4, No. 3, pp 20-24,*

Bonnafous R., Raynaud P. 1980. Le lysozime dans le matériel et les secretion digestives chez le lapin domestique. *Repr. Nutr. Dev., 4:1273-1278.*

Benkerroum N. (2008). Antimicrobial activity of lysozyme with special relevance to milk. *African Journal of Biotechnology Vol. 7 (25), pp. 4856-4867.*

Fleming A.1922. On a remarkable bacteriolytic element found in tissues and secretions. *Proc. Roy. Soc. Ser. B. 93: 306-17.*

Gallois, M., Gidenne, T., Tasca, C., Caubet, C., Coudert, C., Milon, A., Boullier, S., 2007. Maternal milk contains antimicrobial factors that protect young rabbits from enteropathogenic Escherichia coli infection. *Clin. Vaccine Immunol. 14, 585–592.*

Ponti W., Amadori M., Agnoletti F., Bonizzi L., Peri E., Caldora C. 1989. Characterization of some parameters of non-specific immunity in beef cattle (II). *J. Vet. Med. B, 36: 402-408.*

Sensi M., Moscati L., Timi M., Battistacci L. 2006. Evaluation of non specific immunity parameters as prognostic and diagnostic tool in swine pathology: a case reporting. *In Proc.: 19th IPVS Congress, Copenhagen, Denmark, 1, 287.*

Ogundele M.O. 1999. Inhibitors of complement activity in human breast-milk: a proposed hypothesis of their physiological significance. *Mediators of Inflammation, 8, 69–75*

Barret JT. 1983.The Complement System. *In: Barrett JT (ed.). Textbook of Immunology, 4th ed. London: CV Mosby Company; 170–200.*

Chernyshov VP, Slukin I. 1989.Characteristics of local immunity in newborn infants. *Pediatriia* **6**: 20–4

Ogundele M.O. 2001. Role and significance of the complement system in mucosal immunity: Particular reference to the human breast milk complement. *Immunology and Cell Biology 79, 1–10*

Tse H.M., Milton M.J., Piganelli J.D. 2004. Mechanistic analysis of the immunomodulatory effects of a catalytic antioxidant on antigenpresenting cells: implication for their use in targeting oxidation– reduction reactions in innate immunity. *Free Rad. Biol. Med., 36:233-247.*