GENOTYPE AND AGE EFFECTS ON IMMUNITY TRAITS, CORTICOSTERONE AND OXIDATIVE STATUS IN GROWING RABBITS

Abdel-Kafy, E.M.*, Hoda Shabaan M.A., El-Sayed A.F.M., Azoz. A.A.A., Abdel-Latif A. M.
Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.
*Corresponding author: sayedabdkaffy@yahoo.com

ABSTRACT

To investigate the effects of genotype and age on native immunity, corticosterone and oxidative status 60 weaned rabbits were used; 30 Native Middle-Egypt rabbits (NMER) and 30 New Zealand White (NZW). Blood samples were collected and analyzed from each rabbit at 45, 60, and 75 d of age. Native immunity were included count and differential count of leukocyte, 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 was an important source of variation ($p< 0.01$) for leukocytes where NMER was higher than NZW. Effect of age was not significant in Leukocytes. Genotype and age had significant ($p<0.05$) effect in both of Netruphile and Lymphocyte. Netruphile was increased while lymphocyte decreased at 45 d (after 10 d from weaning). Corticosterone levels at 45 days were higher than at others ages in both breeds. Breed significantly influenced Lysozyme and SBA in native immunity traits. NMER rabbits were characterized by a higher lysozyme, an increased SBA and complement when compared to NZW rabbits at different age. Lysozyme and HCA trends were higher at 45d than at 60 d, whereas the SBA increased with age. Reduced plasma levels of ROS in NMER rabbits at 60 and 75 days of age compared to NZW. TAC and ROS were significantly ($p<0.05$) affected by age while breed had no significant effect It can be concluded that NMER rabbits were characterized by of native immunity traits (lysozyme, SBA and complement) when compared to NZW rabbits at different ages.

Key words: Middle-Egypt rabbits, genotype, immunity, corticosterone, oxidative status, age

INTRODUCTION

Appropriate assessment of rabbit welfare should involve multiple indicators such as behavior, physiology, injury, disease and performance (Broom, 1997; Trocino and Xiccato, 2006). In recent years, it has become evident that there is a strong link between behavior, stress, and the neuroendocrine and immune systems (Mann, 2003). We hypothesize that breed composition and age are associated with differences in baseline immune status and corticosterone among different breeds of growing rabbit at different ages. Results of Dal Bosco *et al*. (2009) show that immune and oxidative resistance genotype was an important factor to be considered when a specific rearing system should be adopted. 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). Also, Moscati *et al* 2008 stated that, the immune and oxidative traits of fattening rabbits could be affected by environmental stress (weaning, cage, neighbors). Limited data are available comparing a wide range of baseline immune measures among rabbit breeds across ages. Also, no reports are available on immune measures in native rabbit breeds in Egypt. So, our trial is to investigate the immune traits, corticosterone and oxidative status measures in Native Middle-Egypt rabbits (NMER) and New Zealand White (NZW) across ages during growing period.

MATERIALS AND METHODS

Animal and housing

The trial was carried out at Seds Breeding Station (Bani Suif Governorate in Southern Egypt), 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. Sixty weaned rabbits (35 days of age) of two breeds; 30 kids each from Native Middle-Egypt rabbits (NMER) and New Zealand White (NZW) and sex ratio were 50:50. Each two kids were kept in a cage and fed ad-libitum on a commercial pelleted diet (16.4% crude protein, 13.3% crude fibre, and digestible energy of 10.45 MJ/kg diet**).**

Blood sampling

Blood samples were collected from 30 animals (equal in sex) from each breed at 45, 60 and 75 d of age (n=60). Whole blood samples (about 6 mL per rabbit) were collected via the marginal ear vein. Each sample was divided into two portions placed in tubes with and without anticoagulant. For obtaining plasma the samples were centrifuged at 3000 rpm for 20 minutes and frozen at -20 °C until analysis. After coagulating at room temperature, the serum was extracted by centrifugation and analyzed within 24 hours.

Analytical determinations

Evaluation of the native immune parameters (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. Total count of leukocyte was estimated in fresh blood samples using automatic cells count (AL system, Germany). Differential count of white blood cells was counted by light microscope on oil lens (x100 power). Plasma samples were assayed for corticosterone using Coat-A-Count kit, following the manufacturer's protocol (Diagnostic Products Corp., Los Angeles, CA). Serum lysozyme was measured 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 CH50/50 ml (Barta and Barta, 1993). The levels of ROS are expressed as H2O2 (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

For all traits mean and standard deviation (SD), minimum (Min.) and maximum (Max.) data ware estimated using SAS (1999). A fixed-effects model was used to analyze these variables using the mixed procedure of SAS (1999). The main fixed effects included in the model were breed, age and their interactions. Significance of the differences was assessed by the multiple t-tests. Residuals were tested for departures from assumptions.

RESULTS AND DISCUSSION

The mean Leucocytes count (Table1) was within the range of 5.30 to 9.10 $x10^3/mm^3$ and of 5 to 13 $x\ 10^3/mm^3$ reported Njidda and Isidahomen1 (2011) and by Hillyer (1997) for healthy young rabbits ,respectively . The differential count values of leukocytes (Table 1) were close to those reported by Poljičak-Milas et al., (2009). They found that mean value of the relative lymphocyte, netruphile, and esinophile count in male and female rabbits were between 46.14 to 38.67 %, 60.25 % to 53 % and 0 and 3 %, respectively.

Table 1. Mean and standard deviation of innate immunity and oxidative status parameters (n=60).

Variable	Unit	Mean	SD	Min.	Max.
Leukocytes	$10^3/mm^3$	5.98	0.61	4.5	7.3
Netruphile(N)	%	45.9	4.5	38.0	57.0
Lymphocyte(L)	%	46.1	4.7	33.0	56.0
Monocyte	%	3.91	0.82	2.00	6.00
Esinophile	%	3.06	0.75	2.00	5.00
Corticosterone	ng/ml	35.3	8.0	23.3	56.7
Lysozyme	µg/ml	16.73	5.05	6.4	24.0
Serum Bactericidal Activity(SBA)	%	13.99	2.70	10.0	19.0
Hemolytic complement assay (HCA)	CH50/50 µl	19.1	4.76	10.0	28.5
Total Antioxidant capacity(TAC)	mmol/L	1.43	0.38	0.75	2.00
Reactive Oxygen Substances (H2O2)	mmol /ml	0.333	0.093	0.117	0.481

Mean corticosterone (Table1) was within the range to those reported by Liste *et al*. (2009)(16.1 to 86.5 ng/ml) in young rabbit. Mean lysozyme value (Table 1) was higher than those reported by Carroll and Martinez (1979) (0.85 µg/mL) and Tessler and Weinberg (1975) (4.1 µg/mL) in healthy rabbits. Mean Lysozyme value was close to values stated by Dal Bosco et al (2009) (range from 20.64 to 12.53 µg/mL) while, it was lower than those reported by Moscati *et al.*{ 2008) (27.19 µg/mL). Mean SBA % (Table 1) was lower than those reported by Moscati *et al*. (2008) (42.15 %), but within range of Dal Bosco *et al*. (2009) (10.59 to14.59%). HCA value (Table 1) was within rang of Moscati *et al*. (2008) (0.16 -182.40 CH50/150 µl).

Table 2. Effect of breed and age on leukocytes count and differential count of leukocytes

Variable	Age	Breed (Mean±SE)		P- Value		
		Local	NZW	Breed	Age	Breed X Age
LeukocytesX10^3/mm^3	45	6.3±0.04^a	6.2±0.06^a	0.0010**	0.0737	0.6542
	60	5.8±0.06^b	5.3±0.04^b			
	75	6.2±0.06^a	5.9±0.04^a			
Netruphile (%)	45	42.4±0.23^b	42.3±0.14^b	0.0378*	0.0001**	0.0001**
	60	42.7±0.28^b	49.9±0.40^a			
	75	50.1±0.37^a	48.0±0.29^a			
Lymphocyte (%)	45	49.6±0.25^a	49.4±0.21^a	0.0258*	0.0001**	0.0001**
	60	49.7±0.35^a	42.2±0.39^b			
	75	42.2±0.42^b	44.0±0.29^b			
N\L ratio	45	0.87±0.009^b	0.86±0.006^b	0.102	0.0001**	0.0004**
	60	0.87±0.011^b	1.19±0.023^a			
	75	1.20±0.023^a	1.10±0.014^a			
Monocyte (%)	45	4.2±0.06	3.9±0. 07	0.4514	0.6936	0.6936
	60	3.8±0.13	3.9±0.07			
	75	4.0±0.08	3.7±0.09			
Esinophile (%)	45	3.0±0.08	3.3±0.06	0.1743	0.8135	0.1741
	60	3.1±0.07	2.9±0.03			
	75	2.7±0.04	3.4±0.11			

*P<0.05; ** P<0.01. Within rows, means with different superscripts are significantly different.

Breed was an important source of variation (P< 0.01) for leukocytes (Table 2) where local breed (NMER) was higher than exotic breed (NZW) rabbits. Effect of age was not significant in Leukocytes (Table 2). Breed results are in harmony with Chineke *et al*.(2006) and Burnett *et al* (2006). Effects of age agreed with Olayemi and Nottidge (2007) in rabbits and in the Jack rabbit (Jain, 1986) , while they were in disagreement with, Chineke *et al*. (2006) who reported that age was significant (P<0.05) in leukocytes of rabbits. Breed and age had significant (p<0.05) effect in both Netruphile and Lymphocyte (Table 2). Netruphile was increased while lymphocyte decreased at 45 day (after 10d of weaning); where many searchers reported that post-weaning phase is the most critical period for morbidity and mortality (Maertens and Štruklec, 2006). Low the ratio between netruphile and lymphocytes (N/L) was observed at 45 days compared to those other age (Table 2). N/L ratio is a good index of response to a stressor (Mugnai *et al*., 2011). With advanced age netruphile was increased in both of NMER and NZW breeds (Table 2). Monocyte and Esinophile were not significantly (p<0.05) affected by breed or age or their interaction (Table 2). Burnett *et al*. (2006) reported that age significantly influenced (P<0.05) in netruphile and lymphocyte counts.

Corticosterone levels at 45 days were higher than at others ages in both breeds (Figure 1). Increasing corticosterone level and total leukocyte count (Table 2) at 45 days may conform findings of Poljičak-Milas *et al*. (2009) who reported that while rabbit diseases, stress, and the administration of cortical steroids rarely cause an increase in total leukocyte count, they more frequently cause a change in differential count of leukocytes due to their re-distribution (Jenkins, 2006) and subsequently an altered N/L ration (Table 2).

Native immunity and oxidative status traits were significantly affected by age. Breed significantly influenced Lysozyme and SBA (Table 3), only. NMER rabbits were characterized by a higher lysozyme, an increased SBA and complement when compared to NZW rabbits at differeny age (Table 3). Dal Bosco *et al* (2009) showed that lysozyme values were significantly affected by genotype effect. Also, Mugnai *et al*. (2008) reported that in Leprino breed HCA values increased, while in NZW they decreased with age. Low values of

HCA indicate a high risk of infection. It can also be used in pigs as a parameter for monitoring general health status, including stress assessment (Burger *et al*., 1998).

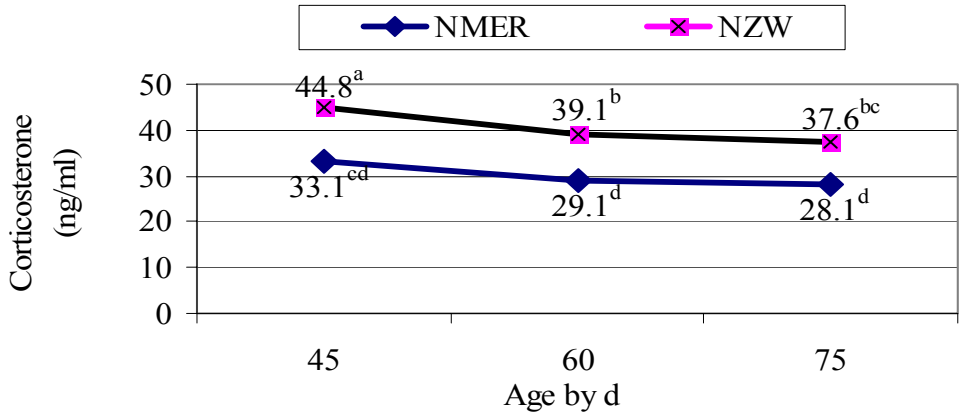

Figure 1: Effect of breed and age on corticosterone level

Table 3. Effect of breed and age on innate immunity and oxidative status parameters

Variable	Age	Breed (Mean±SE)		P- Value		
		Local	NZW	Breed	Age	Breed X Age
Lysozyme (µg/ml)	60	19.3±0.36^{a}	15.9±0.46^{ab}	0.0099**	0.0346**	0.7648
	45	16.5± 0.57^{ab}	12.3±0.45^{b}			
	75	19.1±0.48^{a}	17.0±0.45^{a}			
Serum Bactericidal Activity (SBA) %	45	15.0±0.23^{a}	11.6±0.27^{c}	0.0001**	0.0451*	0.6260
	60	14.0±0.29^{a}	12.2±0.12b^{c}			
	75	16.0± 0.13^{a}	14.0±0.23a^{b}			
Hemolytic complement assay (HCA) CH_{50}/50 µl	45	20.0±0.42^{a}	19.6±0.48^{a}	0.1101	0.0189*	0.3958
	60	18.8±0.52^{a}	14.7±0.56^{b}			
	75	21.3±0.33^{a}	20.1±0.27^{a}			
Total Antioxidant capacity (TAC) mmol/L	45	1.17±0.021^{bc}	1.09±0.029^{c}	0.3385	0.0001**	0.9818
	60	1.43±0.026^{b}	1.38±0.044^{b}			
	75	1.79±0.017^{a}	1.70±0.033^{a}			
Reactive Oxygen Substances H_2O_2 nmol /ml	45	0.399±0.004^{a}	0.364±0.005^{ab}	0.4828	0.0094**	0.2698
	60	0.280±0.009^{b}	0.310±0.008^{b}			
	75	0.296±0.011^{b}	0.349±0.010^{ab}			

*P<0.05; ** P<0.01. As in the other table

SBA is a major parameter of innate immunity and it has been studied as nonspecific host defense mechanisms. Also SBA may play an important role in the initial stages of microbial attack. Table 3 shows that, lysozyme and HCA trends were higher at 45d than at 60 d, whereas the SBA trend was stable and increased with age. The studies of Mugnai *et al*. (2008) and Moscati *et al*. (2008) showed that the HCA of caged fattening NZW rabbits decreased from weaning to 90 d of age while the SBA increased with age. The increased values observed in serum lysozyme and HCA levels at 45 d may indicate an effort to continuously adapt to environmental stress (weaning, change of cage, neighbors, etc) as reported by Mugnai *et al*. (2008) or/and these traits could be associated with inflammatory processes of the intestinal tract (Klass and Neale, 1978).

TAC and ROS were significantly ($p<0.05$) affected by age while breed had no significant effect Reduced plasma levels of ROS in NMER rabbits at 60 and 70 days of age compared to NZW (Table 3). Same trend reported by Dal Bosco et al (2009). Where, ROS generated during biological processes are involved in the pathogenesis of several diseases and various reports have indicated that oxidative stress alters immune competence (Koner *et al*., 1997). Also, increased plasma ROS levels have been frequently associated with the inflammation process (Koner *et al*., 1997). Previous studies (Dal Bosco *et al*., 2002) indicated to a positive correlation between the ROS values and the antioxidant response of the animal.

CONCLUSIONS

NMER rabbits were characterized by a higher native immunity traits (lysozyme, SBA and complement) when compared to NZW rabbits at different age. Results of native immunity traits, corticosterone and oxidative status at 45 d (post weaning) in the present trial confirm previous studies indicating that effort to continuously adapt to weaning, change of cage, neighbors, etc, in addition to being the most critical period for morbidity and mortality is post weaning influence immunity system. To better understanding the dynamics and interaction between such traits with post weaning period should be studied.

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*.

Broom D.M. 1997. Welfare evaluation. *Appl. Anim. Behav. Sci., 54:21-23.*

Burger H.G., Cahir N., Robertson D.M., Groome N.P., Green A., Dennerstein L. 1998. Serum inhibin A and B fall differentially as FSH rises in perimenopausal women. *Clin. Endocrinol., 48: 809-813.*

Burnett N., Mathura K., Metivier K.S., Holder R.B., Brown G., Campbell M.2006. An investigation into haematological and serum chemistry parameters of rabbits in Trinidad. *World Rabbit Sci. 2006, 14: 175 - 187*

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.*

Chineke C.A., Ologun, A.G. and Lkeoli, C.O. 2006. Hematological parameters in rabbit breeds and crosses in humid topics. *Pakistan journal of Biological sciences 9(11):2102-2106.*

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.

Hillyer, E.V. and K.E. Quesenberry. 1997. Ferrets, rabbits, and rodents: clinical medicine and surgery. *W.B. Saunders and Co., Philadelphia, PA, 432.*

Jain, N.C.1986. Hematology of laboratory and miscellaneous animals. In *Schalm's Veterinary Hematology, 4th edn. pp 276–282. Lea and Febiger.*

Jenkins, J. R. 2006. Clinical pathology. In*: BSAVA Manual of Rabbit Medicine and Surgery. (Meredith, A., P. Flecknell, Eds.). British Small Animal Veterinary Association. Gloucester. pp. 45-48.*

Klass H.J., Neale G. 1978. Serum and faecal lysozymne in inflammatory bowel disease. *Gut, 193: 233-239*

Koner B.C., Banerjee B.D., Ray A. 1997. Effects of in vivo generation of oxygen free radicals on immune responsiveness in rabbits. *Immun. Lett., 59: 127-131.*

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.*

Maertens L., Štruklec M. 2006. Technical note: Preliminary results with a tannin extract on the performance and mortality of growing rabbits in an enteropathy infected environment. *World Rabbit Sci.,14: 189-192.*

Mann D.L. 2003. Stress activated cytokines and the heart. *Ann. Rev. Physiol., 65: 81-101.*

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.*

Mugnai, C., Dal Bosco, A., Moscati L., Battistacci L. and Castellini, C. 2011. Effect of genotype and husbandry system on blood parameters, oxidative and native immune status: welfare and implications on performance of organic laying Hens. *The Open Veterinary Science Journal, 5, (Suppl 1: M4) 12-18*

Njidda AA and CE Isidahomen, 2011. Haematology, blood chemistry and carcass characteristics of growing rabbits fed grasshopper meal as a substitute for fish meal. *Pakistan Vet. J., 30(1): 7-12.*

Olayemi, F.O. and Nottidge, Helen O. 2007.Effect of Age on the Blood Profiles of the New Zealand Rabbit in Nigeria. *African Journal of Biomedical Research, Vol. 10; 73 – 76.*

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

Poljičak-Milas, N., I. Kardum-Skelin, M. Vuđan, T. S. Marenja, A. Ballarin-Perharić, Z. Milas. 2009. Blood cell count analyses and erythrocyte morphometry in New Zealand white rabbits. *Vet. arhiv 79, 561-571.*

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

Tessler H.H., Weinberg R.S. 1975. Aqueous and serum lysozyme values in experimental uveitis in rabbits. *Inves. Ophthalm., 12: 953-956.*

Trocino A., Xiccato G. 2006. Animal welfare in reared rabbits: a review with emphasis on housing systems. *World Rabbit Sci., 14: 77-93.*