Effect of seasons on productive performance, metabolic changes and immunity response in rabbits

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ABSTRACT

The present study was carried out to investigate the effect of season on growth performance traits, metabolite assays and gene expression for innate immune in response to vaccination against pasteurellosis. APRI line, weaned females rabbits of were used in this study a local population. Rabbits weaned at 35 days and introduced in the experiment. Weight at 5 weeks considered as the beginning and all rabbits were weighed every 7 days until 12 weeks old. The feed consumption measured at the same weekly interval. Immunity response was studied by vaccination with *Pasteurella multocida* at 8 and 10 weeks of age as challenge experiment and the blood samples taken at 8 , 10 and 12 weeks of age. Gene expressions for interleukin-6 (IL-6) and toll-like receptor-4 (TLR-4) were assayed by Real Time-PCR. Metabolite assays included glucose, triglycerides, and urea nitrogen and blood samples were taken at 8, 10 and 12 weeks of age, respectively. The body weights in winter at W5, W8and W12 of age were higher than those in other seasons. Season had significant ($P<0.05$) influence on daily gain and feed intake. Triglycerides were significantly higher ($P< 0{\cdot}05$) in rabbits during summer. From summer to spring season, the urea values gradually increased significant in rabbits. Vaccination with *Pasteurella multocida* led to an increase in expression for IL-6 in autumn and winter. Expression for IL-6 in spring was lowest the values. Gene expression of TLR4 in rabbits under different seasons had not significantly different. With vaccination by *Pasteurella multocida*, it is recommended by using additives to enhance immunity during summer and spring in rabbit.

Keywords: *rabbit, season, gene expression, immunity, metabolic changes, growth*

Introduction

Domestic rabbits have been considered as one of several alternative species quite suitable source of animal protein in the developing countries (Hanaa *et al*., 2014). Moreover, the rabbit has the capacity to convert both high concentrate feeds and roughage with increased efficiency when compared with large animal species (Hassan *et al*., 1994). Study of genetic and nongenetic factors affecting on productive traits in rabbits is important issue in order to reach highest rates in commercial production (El-Sabrout *et al*., 2014).There is a strong link between stress and immune systems (Mann, 2003). The immune and metabolic rate of fattening rabbits could be affected by environmental stress (Moscati *et al*. 2008, Amici et al., 1998). On the other hand, routine vaccination against pasteurellosis is performed in most rabbit farms despite environmental stress. The prevention is based only on certain technical aspects of hygiene despite environmental conditions and their effects on immunity response in rabbit that may cause vaccination difficult (Kulcsár *et al*., 2008). With climate changes in the last two decades, the growth performances in rabbits need for updating. Also, Limited data are available about immunity response and metabolite assays in rabbit under different seasons. So, our trial is to study the growth performance traits and metabolic changes and to investigate the gene expression for innate immunity in response to vaccination by *Pasteurellosis* under different seasons.

Materials and methods

Atotal number of 384 young rabbits used in this study were born from July 2015 to June 2016, from females of APRI line local population raised in Rabbits Farm of Sakha Station, Animal Production Research Institute, Agriculture Research Center, Egypt. Indoor ambient temperature and relative humidity throughout the experimental period were in summer 28.98°C and 55.3%, in autumn 20.99°C and 63.7% , in winter 16.0°C and 60.4% and in spring were 24.62°C and 51.8 %., respectively. All rabbits were weaned at 35 days and introduced in the experiment and the females and males were equal. Weighed at 5 weeks and considered as the beginning Weight, all rabbits were weighed every 7 days until 12 weeks old. The feed consumption measured at the same previously intervals. Pelleted feed was provided *ad-libitum* and water was always available through automatic nipple drinkers in each wire mesh cage. The chemical analysis of pelleted feed was dry matter 89.6%, protein 16.1%, crude fiber 11.7% and minerals 7.0%. Cages with one rabbit was placed in the experimental building and the ventilation and temperature were natural.

At the day of blood sampling, feeders were closed at 09:00 h. Post-absorption and after 4 to 6 h of feeding

blood sample were randomly collected in 8, 10 and 12 weeks of age from the 30 animals per season. Blood samples were withdrawn from the ear vein into syringes containing EDTA. Blood was centrifuged at 3000g for 15 min. to get plasma stored at –20ºC until assayed parameters of the metabolites. Metabolite assays included glucose, triglycerides, and urea nitrogen. The glucose was assayed by glucose colorimetric detection kit (BioMed- diagnostic). Triglycerides and urea nitrogen concentrations were analysed using enzymatic colorimetric assays from (BioMed- diagnostic) according to Vassault, 1986.

Immunity response was studied by vaccination with *Pasteurella multocida* (*P.m.).*In challenge experiment used 80 rabbits APRI line (n=20 each season). Rabbits at 8 weeks of age was vaccinated (n=10) as treated group and rabbits treated by saline solution (n=10) as control group. Vaccination and treatment by saline were repeated once at 2 weeks interval (at 10 weeks). Blood samples were taken from two groups at 8 (first vaccination and treatment), 10 (after 2 weeks from first vaccination and treatment) and 12 weeks (after 2 weeks from second vaccination and treatment). Seventy two rabbits were killed at 8, 10 and 12 weeks from vaccinated and unvaccinated groups (3 rabbit / time /group) to remove the spleen.

Real Time-PCR

Gene expression for IL-6 and TLR-4 were assayed by Real Time-PCR. RNA was extracted from spleen and for purification used RNeasy Mini Kit. Quantitect SYBR green kit used in Real Time PCR. Sequences of oligonucleotide primers and probes were used in Real Time-PCR shown in table 1. Real time PCR machine was used Stratagene MX3005P. Cycling conditions for Real Time-PCR according to Quantitect SYBR green PCR kits shown in table 2. Amplification curves and ct values were determined by the stratagene MX3005P software. To estimate the variation of gene expression on the RNA of the different samples, the CT of each sample was compared with that of the control group according to the "ΔΔCt" method stated by Yuan *et al*., 2006.

Statistical analysis

For body weight, growth, feed intake and all the parameters taken in account were analyzed using the general linear models procedure of SAS (1999). The model Yij=μ+Si+Xj+eijk included μ= the overall mean, Si =effect of season i=1, 2, 3 and 4, Xj = effect of sex j=1and 2, eijk= residual error term. Effect of sex ignore in the results and focusing on season effect. Duncan test was used to detect significant differences.

Results

Body weights

Body weights (g) in winter at W5, W8, W10 and W12 of age were higher than those in other seasons (Table 3). The high body weight at 5 week of age observed in summer may be explained mainly by the fact that all rabbits weaned during this period were born from females had low weaning survival corroborated with findings of DalleZotte and Paci (2013). They reported that due to the lower litter size of pups born in summer and autumn, their individual weight at weaning were higher than those of pups born in winter ($p<0.001$).

The season effect was studied on the daily gain and feed intakes observed for the whole population between 5 and 12 weeks of age (Table 4). Season had a significant ($P\leq0.05$) influence on daily gain (g) and feed intake (Table 4). The low growth rate observed in summer may be explained mainly by decline in feed intake regularly with the increase of temperature during fattening Table 4. This late type of effect of environmental temperatures was in good agreement with the observation of pervious authors (Poujardieu and Matheron, 1984).Feed conversion in winter was the best comparing with those in others seasons but not significantly (Table 4).

Metabolic changes

The plasma profiles of glucose, triglycerides and urea during all season in rabbits are shown in Table 5. Mean plasma glucose concentrations were not significantly different as affected by season. Triglycerides were higher ($P< 0{\cdot}05$) in rabbits during summer (Table 5). From summer to spring season, urea values gradually significant increased in rabbits (Table 5). These results are similar to those of Ayoub *et al*. (2007) and Okab *et al*., 2008 on rabbits. They attributed these changes to variations in thyroidal activity at different seasons, as exposure to low environmental temperature stimulates the secretion of thyroxine. Thyroid hormones stimulate cholesterol synthesis as well as the hepatic mechanisms that play a central role in regulating fat metabolism (Reddy and Rao 2006). Under normal conditions fatty acids release from adipose tissues far exceeds the rate of fat oxidation, but the excess fatty acids provide a readily available substrate to enable a rapid increase in fat oxidation when required (Romijn *et al*., 1995). The total amount of free fatty acids that can be transported in the plasma is limited because free fatty acids are insoluble and must be transported bound to albumin (Yki-Järvinen, 2010). Secretion of fatty acids as triglycerides therefore serves as an additional potential energy source in the form of circulating lipids (Tuvdendorj *et al*., 2015).

Immunity response

Vaccination with *Pasteurella multocida* (*P.m.)* led to an increase in gene expression for Interleukin-6 (IL-6) an up regulation (1.0 to 0.78 fold) of compared to the uninfected (UI) control in autumn and winter (Figure 1) while expression for IL-6 in spring was

lowest the values (0.42 fold) as shown in Figure 1. The gene expression for IL-6 in season times of vaccination with *Pasteurella multocida* (*P.m.*) revealed a substantial increase in proinflammatory cytokines and an early antimicrobial response as compared to uninfected (UI) control (Schnupf and Sansonetti 2012).

Gene expression of toll-like receptor-4 (TLR-4) in rabbits under different seasons had not significantly different (Figure 2). During the process of intestinal inflammation, intestinal epithelial cells could positively respond to pathogen associated molecular patterns via toll-like receptor (TLRs), which Toll-like receptors (TLRs) belong to the innate immune system and are a major class of pattern recognition receptors representing the first line of the innate immune response (Abrantes et al., 2013). Our results may be due the TLR-4 plays a critical role to regulate inflammatory responses against common bacteria and food antigens (Kajikawa et al., 2005) and the rabbit under same condition during different seasons.

It is summarized that winter and spring had a positive effect on body weight, daily gain (g) and feed intakes. Gene expression of toll-like receptor-4 (TLR-4) in rabbits under different seasons had not significantly different. Vaccination with *Pasteurella multocida* (*P.m.*) led to an increase in expression for interleukin-6 in autumn and winter. It is suggested to use additives for enhancing immunity during summer and spring.

Table 1. Sequences of oligonucleotide primers and probes were used in SYBR Green Real Time-PCR.

Gene	Primer sequence (5'-3')	Reference
GAPDH	TGACGACATCAAGAAGGTGGTG	Schnupf and Sansonetti, 2012
	GAAGGTGGAGGAGTGGGTGTC	
IL6	CTACCGCTTTCCCCACTTCAG	
	TCCTCAGCTCCTTGATGGTCTC	
TR4	GAGCACCTGGACCTTTCAAATAAC'	Kajikawa et al., 2005
	GAACTTCTAAACCACTCAGCCCTTG	

Table 2. Cycling conditions for SYBR green in Real time PCR.

Reverse transcription	Primary denaturation	Amplification (40 cycles)			Dissociation curve(1 cycle)		
		Secondary denaturation	Annealing (Optics on)	Extension	Secondary denaturation	Annealing	Final denaturation
50˚C	94˚C	94˚C	60˚C	72˚C	94˚C	60˚C	94˚C
30 min.	5 min.	15 sec.	30 sec.	30 sec.	1 min.	1 min.	1 min.

Table 3. Body weights (g) in growing rabbits as affected by seasons (Means ± SE).

Season	W5	W8	W10	W12
Summer	578.7^{a}±12.5	1011.5^{ab}±27.4	1277.9^{b}±39.8	1541.8^{b}±56.2
Autumn	519.7^{b}±13.2	929.2^{b}±33.5	1222.3^{b}±49.9	1582.2^{b}±64.4
Winter	581.2^{a}±14.1	1074.8^{a}±43.1	1450.2^{a}±56.9	1880.3^{a}±70.9
Spring	552.7^{ab}±15.1	1067.8^{a}±34.4	1351.8^{ab}±45.6	1572.5^{b}±57.6

^{a,b}Means on the same row with different superscripts are significantly different.

Table 4. Daily gain (g), feed intake (g) and feed conversion in growing rabbits as affected by seasons (Means ± SE).

Season	Daily gain (g)	Feed intake (g)	Feed conversion
Summer	20.69^{b}±0.71	57.67^{b}±1.72	3.82±0.13
Autumn	22.88^{b}±0.83	60.22^{b}±2.04	3.58±0.16
Winter	28.92^{a}±0.99	75.39^{a}±2.5	3.32±0.19
Spring	22.41^{b}±0.79	67.68^{a}±2.04	3.85±0.15

^{a,b} Means on the same row with different superscripts are significantly different.

Table 5. Metabolic changes in growing rabbits under different seasons (Means ± SE).

Season	Glucose (mg/dl)	Triglycerides (mmol/L)	Urea (mmol/L)
Summer	112.1	1.47^{a}	7.40^{b}
Autumn	112.6	1.01^{ab}	7.33^{b}
Winter	123.7	1.15^{ab}	8.05^{ab}
Spring	107.4	0.78^{b}	10.03^{a}
±SE	11.1	0.19	0.64

^{a,b} Means on the same row with different superscripts are significantly different.

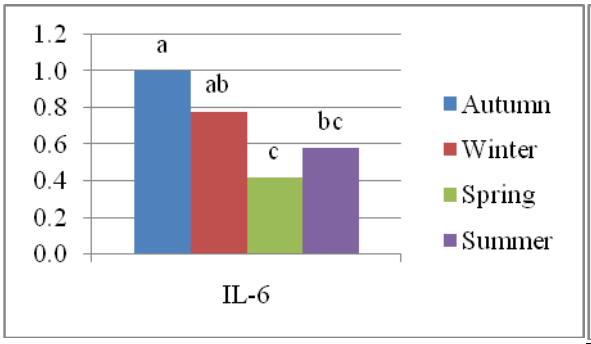

Figure 1: Gene expression of interleukin-6 (TL-6) in rabbits under different seasons

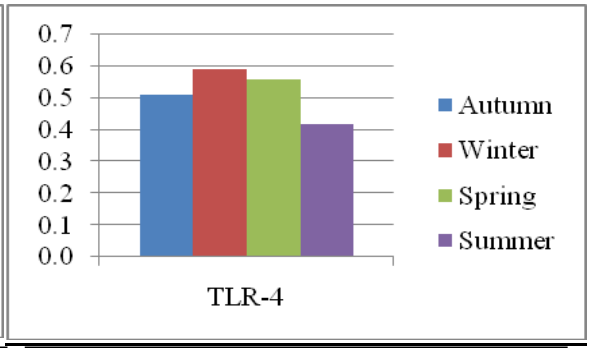

Figure 2: Gene expression of toll-like receptor -4 (TLR-4) in rabbits under different seasons.

References

Abrantes, J., Areal H. & Esteves,P.J. 2013. Insights into the European rabbit (*Oryctolaguscuniculus*) innate immune system: genetic diversity of the toll-like receptor 3 (TLR3) in wild populations and domestic breeds. (). BMC Genetics , 14:73

Amici, A., Canganella F., Bevilacqua, L. 1998. Effects of high ambient temperature in rabbits: metabolic changes, caecal fermentation and bacterial flora. World Rabbit Science, vol.6, 319-324.

Ayoub, M. A. Okab, A. B. & Koriem, A. A. 2007. Effect of seasonal variations on some hematological and plasma biochemical parameters in Egyptian male and female baladi rabbits. The 7th inter. Conf .on Rabbit Prod. In Hot Clim., Ghourdaga, Egypt, 6-9 December, 2007, p. 509-522.

DalleZotte, A. & Paci, G. 2013. Influence of Rabbit Sire Genetic Origin, Season of Birth and Parity Order on Doe and Litter Performance in an Organic Production System Asian-Aust. J. Anim. Sci. 26:43-49.

El-Sabrout, K., El-Seedy, A. Shebl, M.K. Soliman, F.N.K. & El-Sebai, A. 2014. Molecular markers and productive performance relations for line V and Alexandria rabbitsunder Egyptian environmental conditions. Inter. J. Life Sci. Biotechn.Pharm. Res., 3: 345-361.

Hanaa A., El-Raffa, A., Shebl, El-Delebshany, M.K A. & El-Sayed, N. 2014. Genetic evaluation of some economic traits in a maternal line of rabbits. Egypt. J. Poult. Sci., 34: 85-98.

Hassan, N.S., El-Tawil, E.A. Karima, S. & Gad. H.A.M. 1994. Performance of New Zealand White does as affected by different environmental factors. Cahiers Options Mediterraneenns, 1st International Conference of Rabbit Production in Hot Climate, Cairo, Egypt. 8: 271-278.

Kajikawa, O., Frevert, C.W., Lin, S.M., Goodman, R.B., Mongovin, S.M., Wong, V., Ballman, K., Daubeuf B., Elson, G.& Martin, TR. 2005. Gene expression of Toll-like receptor-2, Toll-like receptor-4, and MD2 is differentially regulated in rabbits with Escherichia coli pneumonia. Gene, 344, 193–202.

Kulcsár, G., Fábián, K., Farsang, A. & Virág, Gy. 2008. Evaluation of the protection provided by an inactivated trivalent *Pasteurella Multocida* vaccine against experimental *Pasteurella* infection and Pasteurellosis in commercial environment. 9th World Rabbit Congress – June 10-13, 2008 – Verona – Italy, p 987-992.

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.

Okab, A. B., El-Banna, S.G., & Koriem, A. A. 2008. Influence of environmental temperatures on some Physiological and Biochemical Parameters of New-Zealand Rabbit males. Slovak J. Anim. Sci., 41, (1): 12 - 19

Poujardieu, B., & Matheron, G. 1984. Influence d'une ambiance chaudeethumidesur la croissance de futures reproductrices. 3rd World Rabbit Congress, Rome, I: 107-118.

Reddy, J.K.,& Rao, M.S.,2006 Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. Am.J. physiol. Gastrointest. Liver Physiol. 2006; 290, 852–858.

Romijn, J.A., Coyle, E.F., Sidossis, L.S., Zhang, X.J., & Wolfe, R.R. 1995. Relationship between fatty acid delivery and fatty acid oxidation during strenuous exercise. J. Applied Physiol., 79:1939–1945.

SAS, 1999. SAS user'sguide: Statistical Analysis System Institute, inc. Cary, N.C.

Schnupf, P. & Sansonetti P. J. 2012 Quantitative RT-PCR profiling of the Rabbit Immune Response: Assessment of Acute *Shigellaflexneri* Infection. PLoS One, 7(6): e36446.

Tuvdendorj, D., Zhang, X. Chinkes, D. L. Wang, L., Wu Z., Rodriguez, N. A. Herndon , D. N. & Wolfe

R.R. 2015. Triglycerides produced in the livers of fasting rabbits are predominantly stored as opposed to secreted into the plasma. Metabolism, 64(5): 580–587.

Vassault, A. 1986. Protocol de validation des techniques. Ann Biol Clin. ;44: 686–745.

Yki-Järvinen ,H. 2010. Liver fat in the pathogenesis of insulin resistance and type 2 diabetes. Dig Dis., 28:203–209.

Yuan, J.S., Reed, A., Chen, F. & Stewart, C.N. 2006. Statistical analysis of real-time PCR data. BMC Bioinformatics, Feb 22;7:85.

تأثير الموسم على النمو و التمثيل الغذائى والاستجابة المناعية في الأرانب

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أجريت الدراسة الحالية لدراسة تأثير الموسم على أداء النمو و مقاييس التمثيل الغذائى والتعبير الجيني للمناعة الاساسية والأستجابة للتلقيح ضد لقاح الباستيرلا (*Pasteurella multocida*). استخدمت الأرانب الفطام في هذه الدراسة وكانت من سلالة الأبري. تم فطام الأرانب على عمر ٣٥ يوما وتم أدخلها في التجربة بتسجيل وزنها عند ٥ أسابيع ويتم وزن الأرانب كل اسبوع حتى ١٢ أسبوع من العمر. ويسجل استهلاك العلف كل اسبوع خلال نفس الفترة. وقد درست استجابة المناعه عن طريق التحصين بالباستيرلا في ٨ و ١٠ أسابيع من العمر وتم اخذ عينات الدم عند عمر ٨ و ١٠ و ١٢ أسبوعا. تم قياس التعبير الجينى لجين Interleukin-6 و جين Toll like receptor-4 بواسطة تقنية الـ Real Time-PCR. وشملت الدراسة قياس التمثيل الغذائى والتي شملت الجلوكوز والدهون الثلاثية ونيتروجين اليوريا عند عمر ٨ و ١٠ و ١٢ أسبوعا ، على التوالي. وكانت النتائج: أوزان الجسم في فصل الشتاء في الأسبوع ٥، الأسبوع ٨ والأسبوع ١٢ من العمر كانت أعلى من من المواسم الأخرى. كان للموسم تأثير معنوي ($P < 0.05$) على المأكول و معدل الزياده اليومى فى الوزن. وكانت الدهون الثلاثية أعلى بشكل معنوي ($P < 0 \cdot 05$) في الأرانب خلال الصيف. من موسم الصيف إلى موسم الربيع حدث زياده تدريجيه كبيرة فى قيم اليوريا في الأرانب. الاستجاب المناعية للتطعيم بالباستيرلا أدى إلى زيادة في التعبير الجينى لجين IL-6 في الخريف والشتاء. كان التعبير الجينى عن جين (IL-6) في الربيع أدنى القيم. التعبير الجيني TLR4 في الأرانب تحت المواسم المختلفة لم يختلف اختلافا كبيرا. من خلال دراسة الاستجاب المناعية للتطعيم بالباستيرلا (*Pasteurella multocida*) فمن الافضل استخدام إضافات لتعزيز المناعة خلال فصلى الصيف والربيع في الأرنب.