



EFFECT OF SEASON ON GROWTH PERFORMANCE AND IMMUNITY TRAITS IN RABBITS

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ABSTRACT

The present study was carried out to investigate the effect of season on growth performance traits, leptin hormone concentration and gene expression for innate immunity in response to vaccination against pasteurellosis. A Total of 384 weaned rabbits were used in this study. They were from females of APRI line, a local population. Rabbits weaned at 33-35 days and introduced in the experiment at weaning. Arbitrarily, weight at beginning was considered as the "5 weeks" weight. All rabbits were weighed every 7 days and the feed consumption measured at the same weekly interval until all rabbits were 12 weeks old. Leptin hormone level in serum was assayed. Vaccination with *Pasteurella multocida* was studied in a challenge experiment. Gene expressions for interleukin-6 (IL-6) and toll-like receptor-4 (TR4) were assayed by Real Time-PCR. Parameters of the antioxidant status were included reactive oxygen species, (ROS) expressed as H₂O₂, Lipid Peroxide, expressed as malondialdehyde and glutathione peroxidase. Blood samples were taken at 8, 10 and 12 weeks of age, respectively. The body weights in winter at W5, W8 and W12 of age were higher than those in others seasons. Season had a significant ($P \leq 0.05$) influence on daily gain and feed intake. Season had no significant influence on Leptin hormone level in growing rabbits. Vaccination with *Pasteurellamultocida* led to an increase in gene expression for IL-6 in autumn and winter. Expression for IL-6 in spring has the lowest values.

Gene expression of TR4 in rabbits under different seasons was not significantly different. It is concluded that vaccination by *Pasteurellamultocida* needs some additives to enhance immunity especially during summer and spring seasons.

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 productive traits in rabbits is important issue in order to reach highest rates in commercial production (El-Sabrou et al 2014). There is a strong link between stress, and the neuroendocrine and immune systems (Mann, 2003). Moscati et al (2008) stated that, the immune and oxidative traits of fattening rabbits could be affected by environmental stress. 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. With climate changes in the last two decades and the growth performance traits in rabbits need for updating. Also, limited data are available about immunity response and leptin hormone in rabbit under different seasons. So, the present trail was conducted to study the growth performance traits leptin

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hormone concentration and gene expression for innate immunity in response to vaccination against pasteurellosis under different seasons.

MATERIALS AND METHODS

Experimental Animals

The 384 young rabbits used in this study were born between September 2015 and September 2016, from females of APRI line local population raised in Rabbits Farm of Sakha Station, Animal Production Research Institute, Agriculture Research Center, Egypt. All rabbits were weaned at 33-35 days and introduced in the experiment at weaning. Arbitrarily, weight at beginning was considered as the "5 weeks" weight. All rabbits were weighed every 7 days and the feed consumption was measured at the same weekly interval until all rabbits were 16 weeks old. 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 in the "flat deck" disposition. Ventilation was natural and temperature was not controlled.

Parameters of the antioxidant status

Parameters of the antioxidant status included reactive oxygen species, (ROS) expressed as H₂O₂ (mmol/ml), Lipid Peroxide expressed as malondialdehyde (nmol/ml) and glutathione peroxidase (mU/mL). ROS, Lipid Peroxide and glutathione peroxidase were determined by using commercial kits (Biodiagnostic, Egypt) according to **Koracevic et al (2001)**, **Satoh, 1978** and **Paglia & Valentine (1967)**. Blood samples were taken at 8, 10 and 12 weeks of age, respectively. Serum obtained by centrifugation at 3000 rpm for 10 minutes, and used for determination of reactive oxygen species, (ROS) and Lipid Peroxide. Erythrocyte Lysates buffer was used to prepare red blood cell (RBC) for determination of glutathione peroxidase. Leptin hormone level in serum was assayed by ELISA technique and using DRG Kit following the manufacturer's protocol (DRG instruments GmbH, Germany).

Vaccination Procedure

Vaccination with *Pasteurella multocida* (*P.m.*) was studied in a challenge experiment with 80

rabbits from APRI line (n=20 in each season). The treated group (n=10 rabbits) was vaccinated and 10 rabbit was not vaccinated as negative control group and treated by saline solution at 8 weeks of age. Vaccine and vaccination was repeated once at 2 weeks interval (at 10 weeks).

Blood samples were taken at 8 (first vaccination), 10 (after 2 weeks from first vaccination and at second vaccination) and 12 weeks (after 4 weeks from first vaccination and after 4 weeks from second vaccination). At the same time blood samples were collected from negative control group and treated by saline solution. Sixty rabbits were killed at 8, 10 and 12 weeks from vaccinated and unvaccinated groups (5 rabbit / time /group).

Real time- PCR

Gene expression for IL6 and TR4 were assayed by Real Time-PCR. RNA was extracted from spleen and for purification used RNeasy Mini Kit. Quantitect SYBR green PCR kit used in Real Time PCR. Sequences of oligonucleotide primers and probes used in SYBR Green Real Time PCR were:

Table 1. Sequence of oligonucleotide primers used SYBR Green Real Time PCR

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

Real time PCR machine was used Stratagene MX3005P. Cycling conditions for SYBR green real time PCR according to Quantitect SYBR green PCR kits were:

Table 2. Cycling conditions for SYBR green 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 30 min. | 94°C 5 min. | 94°C 15 sec. | 60°C 30 sec. | 72°C 30 sec. | 94°C 1 min. | 60°C 1 min. | 94°C 1 min. |

Analysis of RT-PCR results

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 (not vaccinated and treated by saline solution) according to the " $\Delta\Delta Ct$ " method stated by Yuan et al 2006.

Statistical analysis

For growth and feed intake study, the parameters taken in account were sex and season of weaning (Summer, autumn, winter and spring). In all cases data were calculated as weights in grams, daily growth rate daily feed intake in grams per day and per rabbit. Main effects were studied by one way analysis of variance and variability expressed generally as residual coefficient of variation or as standard deviation.

RESULTS

Body weights

The results in **Table 3** show that body weights (g) in winter at W5, W8, W10 and W12 of age were higher than those in others season (**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 weights at weaning were higher than those of pups born in winter ($p < 0.001$).

Table 3. Body weights (g) of growing rabbits as affected by season (Means \pm SE).

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

^{a,b} Means in the same column with different superscripts are significantly different.

The season effect was studied on the daily gain and feed intakes observed for the whole population between 5 and 12 weeks of age. Season had a significant ($P \leq 0.05$) 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 effect of environmental temperatures was in good agreement with the observation of previous authors (**Poujardieu and Matheron, 1984**). Feed conversion in winter was the lowest comparing those in others season but not significantly (**Table 4**).

Table 4. Daily gain (g), feed intake (g) and feed conversion in growing rabbits of the as affected by season (Means \pm 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 |

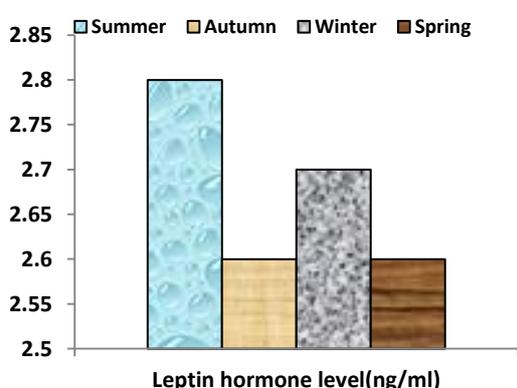
^{a,b} Means in the same column with different superscripts are significantly different.

Leptin hormone level

The results in **Table (5)** show that season had no significant influence on Leptin hormone level in growing rabbits. Leptin is regarded as a signal peptide originating from the adipose tissue informing the CNS about the energy reserves of the body and the adequacy of these reserves for e.g. reproduction (**Friedman and Halaas, 1998**). Although in humans and rodents there is a significant correlation between the body weight and the plasma leptin concentration (**Stamogiannou et al 1997**) but in some on other species there is no significant correlation like in adult Syrian hamsters where the plasma leptin levels do not accurately reflect the body fat content (**Schneider et al 2000**). The minks had clear changes in their plasma leptin levels in the autumn and in the early winter (**Nieminen 2000**). The results of the leptin levels did not show clear seasonal variation which may be due to the leptin level is seasonal adaptation. These results may be attributed to APRI rabbit line that used in the present study were genetically adapted with Egyptian condition.

Table 5. Leptin hormone level in growing rabbits as affected by season (Means \pm SE)

| Season | Leptin hormone level(ng/ml) |
|----------|-----------------------------|
| Summer | 2.8 |
| Autumn | 2.6 |
| Winter | 2.7 |
| Spring | 2.6 |
| \pm SE | \pm 0.09 |

**Fig. 1.** Leptin hormone level in growing rabbits as affected by season

Oxidative profile parameters

Level of hydrogen peroxide (as a source for free-radicals) was significantly affected by season (**Table 6**). Lipid-peroxide as malondialdehyde and Glutathione peroxidase activity concentration was not significantly affected by season (**Table 6**). In the present study three main parameters were used as biomarkers to assess the level of oxidative stress in the organism: the level of cytotoxic and genotoxic Lipid-peroxide as malondialdehyde, Glutathione peroxidase activity as one of the main intracellular free radical scavengers and selenium dependent glutathione peroxidase which is part of the enzymatic antioxidant defense mechanism. Our results support the findings by Kovács et al 2016 who reported that increased Lipid-peroxide referring oxidative stress, presumably because glutathione peroxidase as antioxidant enzyme had the capability to reduce formation of oxygen free radicals and consequently Lipid-peroxide. Increasing ambient temperature in spring seemed the oxidative stress as shown in an increased production of hydrogen peroxide associated with a decrease in glutathione peroxidase that led to damage of the

biological macromolecules and disrupts normal metabolism and physiology (Tse et al 2004) and appeared in increasing Lipid-peroxide (**Table 6**).

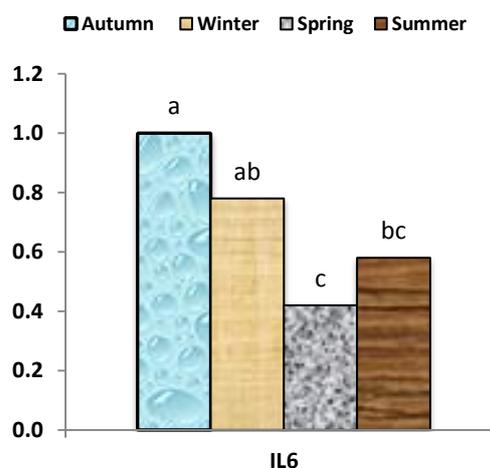
Table 6. Oxidative profile parameters in growing rabbits as affected by season (Means \pm SE)

| Season | Hydrogen peroxide (mmol/ml) | Lipid-peroxide (nmol/ml) | Glutathione peroxidase (U/l) |
|----------|-----------------------------|--------------------------|------------------------------|
| Summer | 3.74 ^{ab} | 3.80 | 786.8 |
| Autumn | 1.79 ^b | 3.26 | 769.2 |
| Winter | 3.02 ^{ab} | 4.21 | 563.1 |
| Spring | 4.66 ^a | 5.23 | 690.8 |
| \pm SE | 0.92 | 0.98 | 282.1 |

^{a,b} Means in the same column with different superscripts are significantly different.

Immunity parameters

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) compared to the control (not vaccinated) in autumn and winter (**Figure 2**) 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 at 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.

**Fig. 2.** Gene expression of interleukin-6 (TL6) in rabbits under different seasons

Gene expression of toll-like receptor -4 (TR4) in rabbits under different seasons did not significantly different (**Figure 3**). During the process of intestinal inflammation, intestinal epithelial cells could positively respond to pathogen associated molecular patterns via toll-like receptor (TLRs), where 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 TLR4 plays a critical role to regulate inflammatory responses against common bacteria and food antigens (**Kajikawa et al 2005**) as the rabbits were under same condition during different seasons.

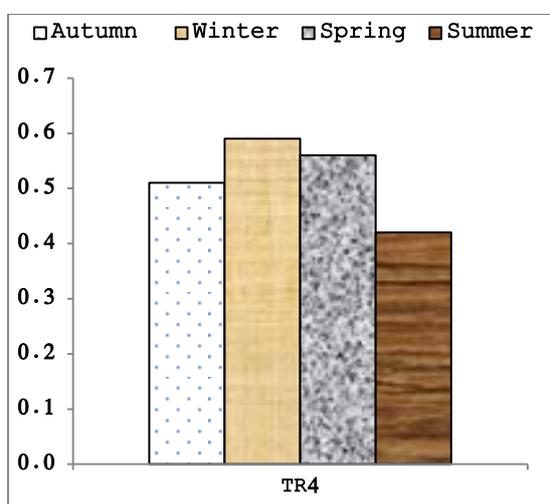


Fig. 3. Gene expression of toll-like receptor -4 (TR4) in rabbits under different seasons

It is summarized that winter and spring had a positive effect on body weight, daily gain (g) and feed intakes. Leptin levels did not show clear seasonal effect. Gene expression of toll-like receptor -4 (TR4) in rabbits under different seasons did not significantly different. Vaccination with *Pasteurellamultocida* (*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.

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