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EFFECT OF EARLY HEAT ACCLIMATION ON IMMUNITY AND SOME INTESTINAL FUNCTIONS AND HEAT SHOCK PROTEIN POST-WEANED RABBITS DURING SUMMER SEASON

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ABSTRACT: The effect of early heat exposure on heat shock proteins and some intestinal functions of weaning rabbits exposed to high ambient temperature was investigated. Forty two New Zealand White (NZW) kits at three days of age were divided into two groups (21 kits/group). The control group (C), kits were kept under normal ambient temperature (25±3°C). The second group (Heat acclimated group) was exposed to high ambient temperature (36±3°C) at day 3post-partum for 1h for 3 consecutive days. All kits were weaned at 28 days of age.

The results indicated that level of Hsp70 was significantly increased in the jejunum and ileum of rabbits in thermal acclimated group compared to control group. Also, early heat exposure in this group improved immune responses as well as, lysozyme and interferon gamma (IFN-γ) activities compared to the control group.

Key Words: Rabbits; Heat Shock; Intestinal Functions And Innate Immunity Functions

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The villous height of the thermal acclimated group was higher than the control group in duodenum and jejunum. Thermal acclimated treatment decreased coliform counts and Clostridium spp in caecum compared to control group. In conclusion, early heat acclimation may improve immune activities and the ability to preserve intestinal integrity of young rabbits when reared under hot condition

INTRODUCTION

Digestive disorders are a major cause of mortality and morbidity in the rabbits around the weaning (Marlier et al., 2003). Susceptibility to digestive troubles may be a consequence of maladjustment of the gastrointestinal function to the transition from doe's milk to a solid diet, probably not adapted to young digestive capacities. Indeed, the digestive tract of rabbits is subject to many changes around weaning. An important anatomical development of the distal relative to the proximal parts of the digestive tract, associated with the development of microbial fermentation and caecotrophy (Lebas and Laplace, 1972).

On the other hand, heat stress causes a series of physiological and metabolic changes in rabbits (Marai et al., 2002). The gastrointestinal tract is particularly responsive to stressors like heat stress, which modify the normal and protective microbiota (Bailey et al., 2004) and decreased integrity of the intestinal epithelium (Lambert, 2009) which in turn can affect its barrier function and the absorption of nutrients, impairing productive performance of animals (Liu et al., 2009). Song et al., (2008) showed that high temperature treatment (40°C, 5 h) induced lipid peroxidation in small intestinal epithelium and a decrease of intestinal immunity in rabbits. Also, Franci et al. (1996) reported how thermal stress treatments diminished the capacity of rabbits' peripheral blood mononuclear cells to proliferate and inhibit the differentiation of B lymphocytes in antibody-secreting cells.

The development of an animal's adaptive capacity does not only have a genetic component, but is also influenced by experiences in pre- and early postnatal life (Star, 2008). Previous studies in different animal species (Vanbesien-Mailliot et al., 2007; Merlot et al., 2008) showed that environmental changes and stressors during certain critical periods in pre- and early postnatal life might exert a major impact on the development of important functional systems, including the thermoregulatory system and immune system.

Moreover, Heat shock proteins70 (Hsp70) considers cytoprotection protein, which

may enhance the tolerance to environmental changes or pathogenic conditions, increase the survival rate of stressed cells, and may also play critical role in preventing the activation of inflammatory cells (Tang et al., 2007). Heat shock proteins (Hsp70) has been shown to protect intestinal epithelial cells from stress factors, this intestinal epithelial protection is associated with restricted bacterial translocation and a reduction in inflammation (Liossis et al., 1997), where it is important location for digestion, absorption and metabolism of nutrients, and easily suffers injury from various stresses (Watanabe et al., 2004).

Therefore, the aim of this study was to determine the effect of heat acclimation on the biological significance of heat shock proteins in postnatal gastrointestinal tract adaptation and their mechanisms of action in gastrointestinal tract disorders and improving immune function in postnatal rabbits.

MATERIALS AND METHODS

Animals and experimental design

This study was carried out at the Rabbits Farm of Sakha Station, Animal Production Research Institute, Agriculture Research Center, Egypt during the period from June to August, 2013. At the beginning of June, forty two New Zealand White (NZW) rabbit kits at three days of age were divided into two groups of 21 rabbits each (2 groups x 7 kits x 3 replicates = 42 rabbits). The first group was kept under normal ambient temperature (25±3°C) as control group. The second group (Heat acclimated group) was exposed to high ambient temperature (36±3°C) at day 3post-partum for 1h for 3 consecutive days by using electric heaters. The exposure time for heat stress was from 11:00 am to 12:00 pm to minimize the effect of circadian rhythm. Also, to avoid the stress of bereavement, the nursing mothers were returned to their newborns after each exposure for all groups.

All kits in both groups were weaned at 28 days of age. Experiment lasted for two weeks after weaning; Rabbits in each group were individually housed in galvanized wire cages provided with feeders and automatic stainless steel nipple drinkers where basal diet and water were offered ad libitum.

The internal minimum and maximum ambient temperatures and relative humidity throughout the experimental period were 25.6°C and 36.5°C, and the relative humidity were 45.5% and 75.5%, respectively. Rabbits were reared under the same managerial and hygienic

conditions as well as fed basal diet contained 2460 ME kcal, 15% CP, 11.17% CF.

Studied parameters:

Biochemical investigations:

In mid- August blood samples were taken from rabbits groups at days 14 post-weaning. Blood samples (twelve representative rabbits from each group) were taken at the time of slaughter into clean sterile tubes with or without heparin before slaughtering time. Non-coagulated blood was tested shortly after collection for estimating blood picture. White blood cells (leukocyte) were counted according to Feldman et al., (2000), and differential counts were determined using the conventional methods. Blood smears (45 samples) were stained for differential leukocyte counts.

Blood samples were let to coagulate and centrifuged at 3500 rpm for 15 minutes and serum was separated and stored at -20 °C untill assay.

Measurement of lysozyme and interferon gamma activities:

Serum lysozyme activity was measured by agarose gel lyses assay according to the method described by Schltz (1987). Briefly, lysophate were prepared by dissolving 1% agarose in 0.06 M phosphate buffered saline at pH 6.3, 500 mg of Micrococcus lysodeikticus in 5ml saline were added to 1 litre of agarose. Plates were poured then 25µl of serum samples and standard lysozyme was put in each well. After 18 hours the cleared zone diameter was measured to both standard lysozyme and serum samples and concentration was estimated while, interferon gamma (IFN-γ) was determined in serum according to (Stachelin et al. 1981 and Kelder et al. 1986, respectively).

SMALL INTESTINE HSP70 LEVEL

Small intestine (duodenum, jejunum and ileum) HSP70 level was measured by enzyme linked immunosorbent assay (ELISA) (Gray et al., 1999). Approximately 0.5 cm3 piece of tissue (duodenum, jejunum and ileum) was homogenized in 1 ml of extraction buffer provided by the kit (Assay Designs Stressgen HSP70 ELISA kit), supplemented with 1 µg/ml aprotinin (a protease inhibitor). After centrifugation at 21,000 g for 10 min, the supernatant was used to estimate proteins by Lowry's method, the rest was diluted by the sample diluent provided by the kit in ratio 1:4 and was used in the assay procedure. HSP70 values were expressed in ng/mg protein.

SMALL INTESTINAL MORPHOLOGY OBSERVATION:

Five rabbits from each group were slaughtered on day 14 post-weaning. The small intestine (duodenum, jejunum and ileum) was removed. Empty small intestinal weight and their lengths were determined. The intestinal density (g weight/length, cm) was calculated. Four cm segments were taken from each portion for histological measurement. These samples were first rinsed with 0.1 M phosphate buffered saline at pH 7.2, and then fixed with 10% neutral formaldehyde. After 24 h, the samples were removed from the fixative, cut into 1 cm^2 sections and stored in fresh fixative. Then embedded in paraffin sectioned at 6 µm thickness and stained with hematoxylin as well as eosin for a light microscopy examination. The villous height and crypt depth were measured based on 15 apparently intact villi from each section according to Yu and Chiou (1997). Morphological indices were measured using image processing and analysis system (Version 1, Leica Imaging System Ltd, Cambridge,UK). The contents of duodenum, were collected, form the slaughtered rabbits, weighed and kept in equal volumes of sterilized physiological saline. They were then individually centrifuged and the supernatant fluids were decanted and used for determination of some digestive enzymes activity of amylase, lipase and trypsin by the method of Marounek et al. (1995)

CAECAL MEASUREMENTS:

Samples of cecum content were taken individually from five rabbits of each group and cecum contents were obtained after slaughtering and filtrated to estimate pH by using pH meter and determination of volatile fatty acids (VFAs) of caecum content that were measured according to AOAC (2000).

CAECUM MICROBIAL COUNT:

Total anaerobic bacterial count and Escherichia coli (E. coli) were estimated according to Collins et al. (1995). Also, Clostridium Spp. and salmonella was examined according to Mackie and Me-Carteny (1953). Technique of colony forming unit (CFU) was adopted and incubation took place at 30 ^{o}C for 2-7 days.

Statistical analysis:

All results were analyzed using the general linear models procedure of SAS (1999). The model was: $Yij= \mu+Gi+eij$; where: μ = the overall mean; Gi = effects of heat treatment and eij = residual error

term. Duncan's multiple range tests was performed (Duncan, 1955) to detect significant differences among means (level of $P< 0.05$ was used).

RESULTS AND DISCUSSIONS

White blood cells counts:

There were significant differences in white blood cells counts, lymphocyte, neutrophil and N / L ratio between groups. Results presented in Table 1 shows that, values of white blood cells count and lymphocyte percentage were significantly higher in rabbits heat acclimated group than control group. While, values of neutrophil percentage were significantly lower in rabbits heat acclimated group than control group. These results are go along with Mahmoud and Yaseen (2005), where they found that feed withdrawal or heat acclimation improved chicks` physiological withstanding of heat stress and a tendency to improved immune response. The lymphocyte is considered the main type of white blood corpuscles and a good indicator of the increase in immune efficiency (Wieslaw et al., 2006). It is understood that the components of immune function are affected by an adverse environment (Shephard, 1998)

Heat stress not only adversely affects production performance but also inhibits immune function (Mashaly et al., 2004) and cause a reduction in antibody production (Zulkifli et al., 2000). This reduction could be indirectly due to an increase in inflammatory cytokines under stress. Moreover, during early heat exposure, the level of inflammatory cytokines increases and heat exposure tends to stimulate the release of IL-6 (Cosio-Lima et al., 2011), which can raise the thermoregulatory set point resulting in increased heat storage. This confirmed the hypothesis that early heat acclimation enhanced the ability to heat resistance and improvements in immune responses.

Also, values of N/L ratio were significantly lower in rabbits heat acclimated group than control group as shown in Table 1. The numeric downward shift in the N/L ratios of early heat acclimated group as compared to the control one is apparently related to increased resistance to bacterial diseases and improvements in immunocompetence (O'Sullivan et al., 1991).

Neutrophils are phagocytic cells designed to defend the organism against infections by bacteria, viruses, or foreign particles, while lymphocytes play an important role in immunity, particularly for the production of antibodies. One of the physiological responses of exposure to

stress is the release of glucocorticoids, causing dissolution of lymphocytes leading to lymphopenia. On the other hand there is an increase in neutrophil release by the bone marrow, thus increasing their number in circulation, under stress (Swenson and Reece, 1996). Our results indicated that this type of stress response was significantly high (increased N:L ratio) in control rabbits and low in those thermally conditioned at an early age. Our findings are in accordance with those reported by Ahmed and Gopal (2012) they showed that early age thermal conditioning was beneficial for the birds to withstand adverse effects of acute heat-stress at marketing age particularly for improving immune function.

On the other hand, beneficial adaptive effect of heat acclimation on the oxidative stress level and inflammation parameters to heat stress can at least partly be due to heat shock proteins (HSP) (Kregel 2002). Heat shock proteins may be important modifying factors in cellular responses to a variety of physiologically relevant conditions such as hyperthermia, oxidative stress and metabolic challenge and modifying factors in acquired thermotolerance (Kregel 2002).

LYSOZYME CONCENTRATION

It is known, lysozyme as an enzyme has the potency of lysis of bacterial cells (Scaman et al., 2006). Lysozyme are detected as widespread enzymes among animals and involved as a natural defense against pathogenic bacteria (Nakimbugwe et al., 2006), enzymatic activity of lysozyme carried out through its lytic function on glycosidic bonds present between N-acetyl muramic acid and N-acetyl glucosamine of cell wall peptdoglycan (Chung and Hankock. 2005). Also, lysozyme are proteins of low molecular weight found in polymorphonucelar leukocytes and mononuclear cells, lysozyme are considered as a member of innate humoral factors and that showed dramatic increase in concentration in response to infection or tissue injury (Weir, 1983).

Table 2 shows the levels of lysozyme, there was significantly increase in the heat acclimated group compared to control group during summer season. In vivo overexpression of the native defense protein lysozyme can protect against acute oxidative stress (Liu et al., 2006). Whereas, the antioxidant properties of lysozymes are partly mediated by a reduction of reactive oxygen species (ROS) levels (Peng et al., 2004).

Moreover, heat acclimation induces beneficial adaptive effects on the responses of oxidative stress and inflammation parameters and suggest that the observed beneficial adaptive effect of heat acclimation on the oxidative stress level caused by the heat challenge can at least partly be due to heat shock proteins (Kaldur et al., 2014).

Activities of interferon gamma (IFN-γ):

The lymphokine (IFN-γ), produced primarily by splenic lymphocytes and natural killer (NK) cells, has been shown to be an important mediator of macrophage activation in controlling a number of intracellular pathogenic (Belosevic, et al., 1988), and immunity to pathogenic infection proceeds in two stages: 1) an early response requiring IFN-γ to limit the growth of the bacteria, and 2) a later adaptive immune response that involves Cluster of differentiation 4 ($CD4^+$)and Cluster of differentiation 8 ($CD8^+$)T cells in protection (Rottenberg, et al., 1999).

Activities of IFN- γ was increased in thermal acclimated group compared to control group during summer season Table (2). These results are in agreement with Iwakabe et al (1998) who reported that NK activity and IFN-γ production from cultured spleen cells was greatly reduced when the animals were under stress. Moreover, IFN- γ is a strong activator of indoleamine 2,3-dioxygenase (IDO) enzyme, which the stimulation of cellular oxidation of tryptophan to kynurenine require superoxide anion for activity, thereby limiting the availability of this amino acid to intracellular microorganisms (Hassanain et al., 1993). Induction of IDO has been positively correlated with inhibition of bacteria growth in vitro (Mehta et al., 1998).

LEVEL OF HSP 70:

An increase in HSP 70 expression occurred at days 14 post-weaning in the duodenum followed by jejunum of thermal acclimated group compared to control group (Table, 2), these results are in agreement with Zhong et al., (2011) in weaning piglets. No significant differences ($P>0.05$) in level of HSP 70 were found among all groups for ileum part, in addition to, HSP 70 expression was less in ileum part compared to the other parts.

Heat shock proteins (Hsp70) play different roles in changes of morphologies and functions of intestine (Pockley et al., 2008). Heat shock proteins (Hsp70) were detected predominantly in the epithelial cells of the whole villi and intestinal

gland. Hsp70 has been shown to protect intestinal epithelial cells from toxic agents and ulcerogenic conditions in gastrointestinal mucosa, where it is important location for digestion, absorption and metabolism of nutrients, and easily suffers injury from various stresses (Watanabe et al., 2004). Also, Hsp70 in the cytoplasm may play an important role in protecting the mitochondria. The mitochondria generate the high amount of ATP. However, under harmful stress condition, mitochondrial ATP production would be impaired in cells (Simmons et al., 2005). It has been confirmed that ATP depletion has detrimental effects on cell homeostasis that include protein aggregation, collapse of the cytoskeleton and loss of ionic balance. Therefore, Hsp70 plays an important role in maintaining mitochondrial integrity, function and capacity for ATP generation. On the other hand, Hsp70 considers cytoprotection protein, which may enhance the tolerance to environmental changes or pathogenic conditions, increase the survival rate of stressed cells, and may also play critical role in preventing the activation of inflammatory cells. Nevertheless, increase HSP70 level in thermal acclimated group may be attributed to facilitate antigen presentation in cells such as macrophages (Theriault et al., 2005), and in activation of innate immunity (Asea et al., 2000). Nevertheless, acclimation to heat improves animal models sustainability to heat and is a leading protective factor against heat stress Yoram, 2008 found that the low stress encountered during the process of acclimation to heat will be reflected by an override in the expression of anti-inflammatory over the pro-inflammatory cytokines.

INTESTINAL CHARACTERISTICS AND MORPHOLOGY:

There were significant differences in the relative weight of small intestine among all groups. The relative weight of small intestine and intestinal density were significantly higher in thermal acclimated group than the control group, while, the insignificant differences were observed in the length of small intestine among all groups Table (3).

Thermal acclimation plays an important role in improving small intestine densities (weight / length). The small intestine density is an indirect measure of the intestinal mass and function (Croom et al., 2006), villi dimension (Palo et al., 1995), and intestinal wall thickness (Trevisi et al., 2009). Low intestinal density in control group might mean low villi height

and width, which results in low intestinal digestive and absorptive capacities (Corless and Sell, 1999). High ambient temperature leads to generation of free radicals, which can induce lipid peroxidation and thereby damage cell structures (Altan et al., 2003). Maintenance of normal morphology and structural integrity of the small intestine are imperative for preventing bacterial translocation from the intestinal tract. Also, heat stress could exert deleterious effects on the absorptive epithelium of the intestine, resulting in reduction in villus height and crypt depth (Yamauchi et al., 2006), as well as, heat stress is associated with intestinal irritation.

As shown in Table 3, the results showed that heat acclimated group has significantly improve in the villus height and crypt depth values of duodenum and jejunum when compared to the control group. These results are agreed with the findings of Wu et al. (1996) in rabbits and Uni et al. (2001) in broiler chickens who suggested that heat conditioning at d 3 post-hatch alters T_3 hormone levels, which affects the small intestine by changing cell proliferation and enzyme activity. This relationship leads to the conclusion that changes in small intestinal morphology and enterocyte dynamics could result directly from early thermal conditioning. Heat acclimation facilitated the survival and proliferation of intestinal mucosal cells and that HSPs synthesis from this treat maintains the mucosal integrity and defenses (Liu et al., 2002).

There were no significant differences between the treatments in the ileum measurements as shown in Table 3. There were slightly increased in all ileum measurements for heat acclimated group compared to the control group. There are several possible reasons why ileum structure was unchanged in this experiment, including the resistance of the ileum to structural changes compared with other regions of the small intestine and possibly, the high temperature stress was not affect on it. In poultry, Yamauchi et al. (1996) indicated that morphological changes in response to stressors occur more rapidly in the proximal two-thirds of the small intestine than in the ileum.

As shown in Table 3, there was insignificant increase in villus height/crypt depth ratio of all intestine parts in heat acclimated group compared to the control group. It has been cleared that the villus height/crypt depth ratio is a valuable criterion for estimating the digestive capacity of the small intestine and decrease in this ratio is considered to be

deleterious to digestion and absorption and vice versa (Pluske et al., 1997).

Pancreatic Enzyme Activities: There were significant differences in the relative weight of pancreas between groups as shown in Table 4. The relative weight of pancreas was significantly higher in thermal acclimated group compared to the control group.

Effect of thermal acclimation on the pancreatic enzyme activities are presented in Table 4. It is clearly observable that, the activity of amylase and trypsin enzymes of the pancreas was significantly higher in the group of thermal acclimation than the control group. Thus, high pancreatic amylase activity indicates that pancreas plays an important role in the regulation of the levels of intestinal amylase and glucose supplied to achieve energetic requirements under these conditions.

Concerning lipase enzyme the current findings indicated that, its activity was slightly affected with treatment. Where, the non significant differences were noted between the groups. It has been suggested that the amount and type of triglycerides in the diet not the environmental conditions regulate pancreatic lipase activity and the mechanism is still unclear (Ricketts and Brannon, 1994).

Trypsin enzymes play a central role in protein digestion and trypsin activity has been positively correlated with digestion efficiency and growth in rabbits as reported by Debrey et al., (2003) who found that relative activities of trypsin and chymotrypsin increased after weaning. Results of trypsin enzyme activity showed that, it was elevated significantly with thermal acclimation compared with the control group. In addition, it is mentioned that enhancement of activities of digestive enzymes by heat acclimation as previously reported by Abdel-Kafy et al., (2008) who suggested that neonatal heat exposure may improve the efficiency of enzymes activity in the gastrointestinal tract of rabbits. This result could also increase nutrient digestibility and improve the regulation and stabilization of the gut microbiota. Moreover, studies reported that the induced high expression of heat shock proteins offered a certain degree of protection for pancreatic tissues and reduced the inflammation in the pancreas (Takacs et al. 2002). On the other hand, the cell adaptive response, as elevated HSPs have a protective effect on the pancreatic tissue (Seo et al. 2005)

CAECUM TRAITS:

The pH values in the caecum depended, to a certain extent, on the level of volatile

fatty acids (VFA) in the caecum. Also, according to Veselin et al.,2004 , pH in the caecum varies in the range 6.0-7.4 and depends on the type of the diet, the level of degradability of nutrients in the stomach and small intestines, the caecum wall movement and the level of the carbohydrate atoms. The constantly incoming alkaline from the small intestines to the caecum maintains pH, which is close to neutral and creates favorable conditions for the development of the microorganisms in the caecum.

The pH of the caecum contents were significantly affected by treatments, pH of the caecum contents was lower in early heat acclimated group than control group (Table 5). Our results agree with those of Guedes et al., (2009).

Thermal acclimation leads to significantly increase in the concentration of total VFA of this group compared to control group as shown in Table 5. Whereas, heat stress in lactating animals results in dramatic reduction in roughage intake, gut motility and rumination which in turn contribute to decrease volatile fatty acid production and may contribute to alteration in butyrate: propionate ratio. (Collier et al., 1982). The exposure to heat stress for 2 or 14 days induced a dramatic decrease of VFA concentration in the caecal content and similar trend was observed for acetic, propionic and butyric concentration in rabbits (Amici et al., 1998).

The role of VFA, in particular increased the amounts of butyrate are reported to be a limiting factor for E coli proliferation in rabbits (Morisse et al., 1985), whereas the anti-inflammatory effect of butyrate and its specific role on mucosal integrity in rats were reported by Andoh et al. (1999). It observed a negative correlation between pH and butyrate proportion (Amici et al., 1998). Also, it has been shown that increased caecal VFA and low pH inhibit pathogen's proliferation in the caecum (Prohaszka 1980). Furthermore, early thermal acclimation might be play role result in more butyric acid than propionic acid as an end product of cecum fermentation, this may be important in preventing enteritis (Cheeke, 1987). Better gut health in the young rabbits associated with high caecal VFA level and low pH and NH3 concentration (Gidenne et al., 2004).

Caecum microbial counts:

Data in Table 5 shows that rabbits treated with early heat exposure had significantly lower counts of total anaerobic bacteria, Escherichia coli and Clostridium spp. than the control group. These results are in

agreement with Amici et al., (1998) who found that the microbial population, total anaerobes counts and clostridia result significantly high values in heat stressed group compare to control group. As a result of heat acclimation could be attributed to the reduction of harmful caecum microbial counts and influence in the intestinal microbiota by changing the intestinal environment to be unsuitable or un appropriate conditions for pathogenic bacteria. Also, this may be due to suppress the growth of bacteria as a result to moderate pH of the media (Yeo and Kim, 1997). Furthermore, Morisse and Cheeke (1986) showing digestive troubles (diarrhoea) in rabbits that had high pH values of the caecal content (above 7.0), these conditions are unfavourable for the caecal bacterial flora and allow the development of bacteria, as E coli and clostridia, which be able to produce toxins that are lethal for rabbits. Also, Morisse et al., (1985) observing no signs of disease in rabbits with an abnormal growth of caecal clostridia and E coli suggested that the toxin production is inhibited by low pH values (5.8) and the caecum was colonized by non pathogenic strains.

CONCLUSIONS

Early thermal acclimation plays an important role in modulate and improving small intestinal morphology, digestive enzyme activities and immunostimulation for better rabbits farming.

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Table(1): Effect of early neonatal heat exposure on white blood cells of rabbits during summer season

Item	Control group	neonatal heated group	SE
white blood cells × 10^3 /mm^3	5.64 [b]	6.84 [a]	± 0.11
Lymphocytes %	62.02 [b]	69.88 [a]	± 1.01
Neutrophils %	35.15 [a]	24.70 [b]	± 0.87
N / L ratio	0.56 [a]	0.35 [b]	± 0.01

a, b, :Means in the same row bearing different superscripts are significantly different.

Table(2): Effect of early neonatal heat exposure on Lysozyme, Interferon gamma (INF-γ) and HSP70 levels of rabbits during summer season

Item	Control group	neonatal heated group	SE
Lysozyme (mg/l)	38.60 [b]	48.60 [a]	± 1.82
INF-γ (pg/ml)	385.70 [b]	495.40 [a]	± 9.91
HSP70 level in duodenum (ng/mg protein)	2.99 [b]	4.18 [a]	± 0.17
HSP70 level in jejunum (ng/mg protein)	2.63 [b]	3.53 [a]	± 0.13
HSP70 level in ileum (ng/mg protein)	1.65	1.39	± 0.44

a, b, :Means in the same row bearing different superscripts are significantly different.

Table(3): Effect of early neonatal heat exposure on intestinal characteristics and morphology of rabbits during summer season

Item	Control group	neonatal heated group	SE
Relative small intestine wt. (g)	30.70 [b]	35.50 [a]	± 0.83
Small intestine length (m)	1.62	1.56	± 0.08
Small intestine density (g/m^{-1})	19.09 [b]	24.19 [a]	± 1.85
Duodenum (μm) Villus height	257.80 [b]	392.30 [a]	± 20.03
Crypt depth	138.70 [b]	178.60 [a]	± 6.83
Villus height/crypt depth ratio	1.89	2.21	± 0.12
Jejunum (μm) Villus height	252.50 [b]	363.60 [a]	± 19.83
Crypt depth	169.40 [b]	203.00 [a]	± 19.82
Villus height/crypt depth ratio	1.63	1.84	± 0.21
Ileum (μm) Villus height	235.30	249.50	± 8.39
Crypt depth	181.90	192.10	± 10.27
Villus height/crypt depth ratio	1.31	1.36	± 0.10

a, b, :Means in the same row bearing different superscripts are significantly different.

Table(4): Effect of early neonatal heat exposure on relative pancreas weight and pancreatic enzyme activities of rabbits during summer season

Item	Control group	neonatal heated group	SE
Pancreas %	0.28 [b]	0.31 [a]	± 0.01
Amylase (U/g)	20.79 [b]	29.63 [a]	± 1.11
Lipase (U/g)	51.50	52.68	± 1.58
Trypsin (U/g)	60.89 [b]	70.75 [a]	± 1.88

a, b, :Means in the same row bearing different superscripts are significantly different.

Table(5): Effect of early neonatal heat exposure on cecum traits of rabbits during summer season

Item	Control group	neonatal heated group	SE
Cecum pH	6.45 [a]	5.96 [b]	± 0.06
Total VFA (mmol/l)	62.74 [b]	72.47 [a]	± 1.29
Escherichia Coli (× 10^2 CFU/g	707.10 [a]	595.10 [b]	± 11.38
Total anaerobic bacteria (× 10^6 CFU/g)	5.97 [a]	5.01 [b]	± 0.08
Clostridium spp (× 10^3 CFU/g)	2.84 [a]	1.67 [b]	± 0.17

a, b, :Means in the same row bearing different superscripts are significantly different.

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الملخص العربى

تأثير الاقلمة المبكرة للحرارة على المناعة وبعض وظائف الامعاء وبروتينات الصدمة الحرارية للأرانب بعد الفطام خلال موسم الصيف

عبد العزيز سيد عثمان البدرى ، فضيلة محمد عيسى ، أمل مغاورى هيكل ،آيات رجب

قسم بحوث تربية الأرانب والرومى والطيور المائية، معهد بحوث الإنتاج الحيوانى، مركز البحوث الزراعية، الدقى، جيزه، مصر

تم دراسة تأثير المعاملة الحرارية المبكرة للأرانب حديثي الولادة على بروتينات الصدمة الحرارية و وبعض وظائف القناة الهضمية للارانب المفطومة المتعرضة لبيئة حارة. تم تقسيم اثنين وأربعين ارنب نيوزيلندا أبيض الى مجموعتين (21 ارنب / مجموعة). المجموعة الاولى تعتبر كنترول ، تم الاحتفاظ بها تحت درجة الحرارة العادية (٣± ٢٥ درجة مئوية). بينما تعرضت المجموعة الثانية (المجموعة المتأقلمة) لارتفاع درجة الحرارة المحيطة (٣ ± ٣٦درجة مئوية) في اليوم الثالث من الولادة لمدة ٣ أيام متتالية باستخدام الدفايات الكهربائية. تم فطام كل المجاميع عند اليوم ٢٨ من العمر.

أشارت النتائج إلى أن مستوى HSP70 زاد زيادة كبيرة في الصائم واللفائفي للأرانب في مجموعة التأقلم الحراري مقارنة بمجموعة الكنترول. في حين تحسنت مجموعة التأقلم الحراري للاستجابات المناعية وكذلك نشاط الليزوزيم والانترفيرون مقارنة بمجموعة الكنترول. كذلك تحسنت مقاييس خملات الاثنى عشر والصائم فى مجموعة التأقلم الحراري مقارنة بمجموعة الكنترول.ايضا

انخفضت بكتريا القولون والكلوستريديوم spp في الأعور فى مجموعة التأقلم مقارنة بالمجموعة الكنترول .

واظهرت النتائج إلى أن HSP70 لديه وظائف مختلفة في تحسين مقاييس الخملات بالغشاء المخاطى لأمعاء الأرنب بعد الفطام حيث أن HSP70 له دور مهم فى حماية التركيب الهستولوجى والوظيفي للأمعاء .

لذ توصى الدراسة بان تعرض الارانب حديثي الولادة للحرارة ادى الى تحسين وتنبية الجهاز المناعى وكذلك تحسين القدرة على الحفاظ على سلامة الأمعاء الدقيقة للأرانب المفطومة عندما تربى تحت ظروف بيئية حارة. وربما يرجع ذلك للحد من البكتيريا المسببة للأمراض مثل بكتيريا القولون وكلوستريديوم spp.