

INFLUENCE OF SHORT HEAT EXPOSURE, BALANCED FEED RESTRICTION AND ACETIC ACID ON GASTROINTESTINAL TRACT AND MORTALITY DURING POST-WEANING IN RABBITS WITH EMPHASIS IN HEAT SHOCK PROTEIN

By

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Abstract: *This study was aimed to evaluate whether induction of heat-shock protein 70 and 90 by short heat exposure, balanced feed restriction or acetic acid supplementation is protecting against intestinal injury that occur during weaning. Kits were weaned at 30 days of age and divided into 4 groups (each group 20 kits) as following: 1st group: kits were kept as control (Control); 2nd group: kits were fed restricted diet ($\approx 75\%$ ad-libitum diet) during 1st week post weaning (Restriction); 3rd group: kits were exposed to high ambient temperature ($34 \pm 2^\circ\text{C}$) for 1 hour (Heat); 4th group: kits were supplemented by 0.5 % acetic acid in drink water (Acetic).*

Quantity of both mRNA heat shock protein families 70 (HSP70) and 90 (HSP 90) in jejunum was recorded with fed restricted diet compared with the other groups. Value of mRNA 90 only in jejunum of heat and acetic acid supplemental groups was higher than that control. Activities of amylase, protease enzymes in small intestine contents of heat exposure were higher while, lipase was lower compared with those in control group. The significant reduction in N/L ratio in both of feed restriction and heat groups may be a good indicator of the increase of primary immunity. Villous height and crypt depth (μm) in mid-jejunum in control group were lower than that in other groups. In summary: Balanced feed restriction applied immediately thereafter the weaning (for one week) resulted in increasing expression of both HSP 90 and 70 which reflected on protection of the villous-crypt structure and immune function against some forms of stress. Feed restriction and shot heat exposure and acetic acid supplementation decreases the post weaning mortality rate (%) post weaning to 13.3, 13.3, and 20 respectively; vs. 33.3 in control.

INTRODUCTION

Mortality rate is about 30% for young born throughout the year and the sever mortality is happened post-weaning during (5-10 weeks of age) as reported by Nikkels *et al.*, (1976). After weaning, non-specific enteropathies arise in rearing rabbit. These troubles lead to a dysfunction in intestinal tract. But few studies have been related to maturation of the digestive system in rabbit as reported by Laurence *et al.*, (2003). In pigs many studies reported that there is a reduction in villous height and either an increase or decrease in crypt depth after weaning (Van Beers-Schreurs *et al.*, 1998). This is possible due to contributions of local inflammatory reactions to villus-crypt during the weaning (McCracken *et al.*, 1999). Also, the activity of the digestive enzymes in pancreatic tissue is low after 5 days post-weaning due to interaction with other factors which may increase the risk of developing post-weaning diarrhea (Hedemann and Jensen 2004). On the other hand, Stressors like increased temperature, nutritional deficiencies and other forms of stress which cause increase synthesis of heat shock protein (HSP) (Rotii-Roti and Laszla, 1987). Heat shock proteins are a set of well classified according to their molecular mass, which ranges from to >Thermal pre-treatment in experimental sepsis-induced acute organ injury KDa (David and Grongnet, 2001) associated with the synthesis of heat shock proteins (HSP), reduces organ damage and enhances animal survival (Villar *et al.*, 1994). Also, heat stress applied immediately thereafter the administration of bacterial endotoxin resulted in increasing in expression of HSP 70 that provides protection against acute intestinal inflammation (Stojadinovic *et al.*, 1997). These are related to the generation of heat shock protein transient, even if the exposure to stress is for long period. As a continued presence of heat shock proteins would adversely influence protein homeostasis and a variety of intracellular functions (Shi *et al.*, 1998). Role of HSP is appearing in the induction of gene transcription for proteins with the capacity to stabilize and re-fold proteins, thereby re-establishing the balance between protein synthesis, assembly and degradation (Nover, 1991).

Organic acids (Formic acid, Lactic acid, Acetic acid and Propionic acid are recognized as one of the best alternatives of antibacterial growth promoters in animal feed. They can also be delivered in the drinking water. Organic acids in the popeline help kill bacteria, increasing digestibility of proteins and regulating the micro flore in the gut (Philipsen, 2006).

This study was aimed to evaluate either induction of heat-shock protein 70 and 90 by heat exposure, feed restriction or acetic acid can provide a protection against intestinal injury that occurs during weaning.

MATERIAL AND METHODS

This work was carried out in commercial farm located in Duika area, Cairo Governorate, Egypt. This work was continued for 4 weeks in summer seasons (15th May to 15th June, 2007). A total number of eighty New Zealand White (NZW) rabbit kits (weaned at 30 days of age) with average weights ($606.9.2 \pm 26.6$ g) were divided into 4 groups (each group 20 kits) as following: 1st group: kits were kept as control (Control); 2nd group: kits were fed restricted diet ($\approx 75\%$ ad-libitum diet) during the first week post weaning (Restriction). Quantity of feed restriction was calculated as a relative to quantity of feed ad-libitum consumption according to Okerman (1994); 3rd group: kits were exposed once to high ambient temperature ($34 \pm 2^\circ\text{C}$) for 1 hour using electric heaters supplied with thermostat and thermometer (Heat); 4th group : kits were supplemented by 0.5 % acetic acid in drinking water (Acetic) for one week. Feed intake was recorded during the first week post weaning. Weight and mortality % of kits were recorded weekly from weaning until the 4th week post-weaning.

Five kits from each group were sacrificed at 1st week post-weaning. Blood samples were collected during sacrificing into tubes heparinized to determine blood picture and differential count of leukocyte (%). Gastrointestinal removed for collecting contents of small intestine and caecum into tubes and stored at -80°C until enzymes assay. Small intestine length was measured by a ruler according to the method of Steeb et al. (1991) and calculated as relative to body weight (Van-Soest, 1982). Mid-jejunum samples of the four kits from each group were pooled in epen dorff tubes and kept in liquid nitrogen until detection and determination mRNA heat shock protein (HSP) families 70 & 90.

The mRNA heat shock protein (HSP) families 70 & 90 were detected and determinated by Reverse Transcription Polymerase Cycle Reaction (RT-PCR) according to manufacturers kit ABgene UK as following: (1) Extraction of total RNA from samples was carried out by using kit (QIAGEN, 1999) for total RNA isolation and quantified by spectrophotometer (260 nm). (2) 1mg of total RNA was reverse-transcribed using kit (One-Step RT-PCR ABgene UK) in a total reaction volume of 50 μl . (3) 1 μl of reverse transcription product (cDNA) was amplified by using amli-taq DNA polymerase (ABgene, UK) and 0.5 μl & 1 μl each of HSP 70 & 90, respectively from forward and reverse primers. (4) The PCR cycle consisted of annealing at 65°C for 30 sec. and primers extenuation at 72°C for 1 min for a total of 35 cycles followed by 72°C for 5 min as final extenuation. (5) Electrophoresis of PCR products through 1.5% agarose gel stained with ethidium bromides.

Jejunal morphology was measured (Height villous and crypt depth) in samples of mid-jejunum 1st week of age. Immediately after slaughtering, samples of mid-jejunum were fixed in formalin buffered then they were embedded in paraffin wax, sectioned (5 μ) and stained by haematoxyline and eosin (Drury, *et al.*, 1967). Height of villi (crypt and villus) in jejunum samples was determined by using light microscope and micrometer lens and slide.

All enzyme activities were assayed in contents both of small intestine and caecum colorimetrically as following: amylase (U/ml) was assayed using starch according to Somogyi (1952). Protease (U/ml) was assayed by using azo-casein as a substrate according to Chopra and Mathur (1983). Lipase (U/ml) was assayed by colorimetric technique using kit of Biodiagnostic (Egypt).

Statistical Analysis

All results were analyzed using the general linear models procedure of SAS (1999). Means were compared ($P < 0.05$) using Duncan's multiple range test, (Duncan, 1995). The model $Y_{ij} = \mu + G_i + e_{ij}$. That included G_i = effect of treatments, e_{ij} = residual error term and μ = the overall mean .

RESULTS AND DISCUSSION

Heat shock protein

The highest value for both mRNA heat shock protein families 70 (HSP70) and 90 (HSP 90) in jejunum was recorded with kits fed restricted diet compared with the other groups (Figure 1&2). This may be due to feed deprivation was induced a twofold increase in mRNA for HSP 90 (Gasbarrini *et al.*, 1998). The value of mRNA 90 in jejunum of heat and acetic acid supplement groups was higher than that control (Figure 2). HSP 90 has a role as a housekeeping protein for cell growth and differentiation (Tanguay *et al.*, 1993). The value of mRNA HSP 70 in the heat group was nearly equal to control group (Figure 1). However, the hyperthermia-induced peak increase in mRNA HSP 70 at 2 hrs preceded that of HSP 70 induced at 4 hrs (Stojadinovic *et al.*, 1997).

Small intestine and caecum enzymes

Activities (U/ml) of amylase and protease enzymes in small intestine contents for heat exposure group were higher while, lipase was lower as compared with those in control group (Table 1). Exposure to heat caused changes in the activity of small intestine enzymes. The character of these changes depends on the intensity of heat stress and its duration (Rakhimov

et al., 1976). Uni *et al.*, (2001) reported that mRNA expression of jejunum enzymes increased after short-heat exposure in chicks and this may be translated to the new enzymes synthesis.

At day 7 after feed restriction activity of amylase was higher than that control while, lipase was lower than one. The same trend was founded by Marion *et al.*, (2003) who, reported that at 1st week after weaning, the activity of amylase was higher, and lipase activity was low in piglets which consumed more feed than those consumed less feed.

Hematological responses

Insignificant differences were observed between groups in all hematological parameters (Table 2). The decrease of hematocrit and hemoglobin level in feed restriction group than other treatments may be due to decline of total protein and albumin in plasma as a result of feed restriction as reported by Pond *et al.* (1986).

Differential count of leukocyte

Significant differences were observed in neutrophils in restriction group compared with other groups (Table 3). The significant reduction in N/L ratio in both restriction and heat groups may be a good indicator of the increase of primary immunity (Table 3). This confirms the findings of Good and Lorenz (1992) and Heydar *et al.* (1993). They reported that balanced feed restriction lead to maintain vigorous immunologic functions. Neutrophils number in short-heat exposure group was lower than that control. Whereas, Stojadinovic *et al.*, 1995 reported that numbers of neutrophilic infiltration (neutrophils) were well correlated in non-heated ($r = 0.72$) but not in heated groups ($r = -0.16$) in histological assessment of mucosal injury. These results could be explained that mRNA HSP 70 and 90 syntheses (Figure 1 and 2) may protect immune function against some forms of stress as reported by Ciavarra and Simeone (1990).

Feed restriction led to reduce in small intestine length (mm) and their relative length (Table 4). This result is similar with results finding by Palo *et al* 1995, who reported that feed restriction resulted in a reduction of the size and cell number in jejunum, but gastrointestinal tract segments were less affected by feed restriction and responded more quickly to re-alimentation than the whole body in rat (Anugwa and Pond ,1989) and broilers (Palo *et al* 1995).

There were no significant differences between groups (Table 4) in the small intestine and caecum pH. Villous height and crypt depth (μm) in mid-jejunum were lower in control group than that in other groups (Table

4). The significant differences in villous height and crypt depth at 1st week post weaning (Table 4) may be due to the marked changes occurred post-weaning such as villous atrophy, crypt hyperplasia and increased cell permeability at the same time, there are replacing to these atrophy and dystrophy cells by cells formed from crypts (at the base of the villi) as reported by Van-Dijk *et al.* (1999). Increasing the villous height and crypt depth in heat group may be due to significant decrease in the number of infiltrating neutrophils and increase expression of HSP 70 which provide the protection against acute intestinal inflammation (Stojadinovic *et al.*, 1997). Also, the linear relationship between the jejunal villous height, crypt depth and expression of HSP 90 (David *et al.*, 2002) was higher in heat and acetic groups than that control group. (Figure 1).

Mortality rate:

Restriction of feed decreases the mortality rate of kits rabbits (Table 5). This is a harmony with suggest of Nikkels *et al.*, (1976), who reported that restriction of food decreases the mortality rate from enteritis among young rabbits. Early heat exposure can provide protection against lethal stimulus in rat exposed to a bacterial endotoxin and that the mechanism of protection may be related to the attenuation of plasma interleukin-1 beta concentrations (Chu *et al.*, 1997). These results indicate that HSP plays a central role in the protection of cells, tissues or organs subjected to various types of stress (like weaning shock) as reported by David *et al.* (2002). Reducing the mortality during 1st, 2nd and 4th week post weaning in acetic acid may be confirm the acetic acid effect as antibacterial growth promoters in animal feed (Philipsen, 2006).

In conclusion, feed restriction applied for one week after weaning increase the expression of both HSP 90 and 70 that reflected in protection of villous height and crypt depth. Neutrophils/Lymphocytes ratio was significantly reduced (as increase of primary immunity) in both of restriction and heat groups.

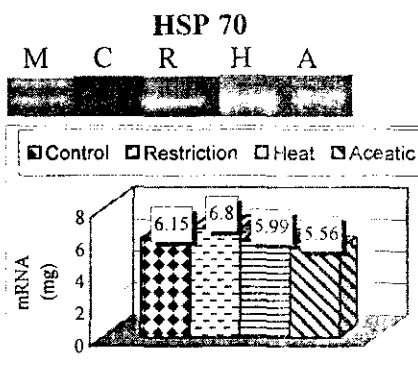


Figure 1: mRNA value of HSP 70 in jejunum as affected by feed restriction, heat exposure and acetic acid supply in NZW rabbit kits

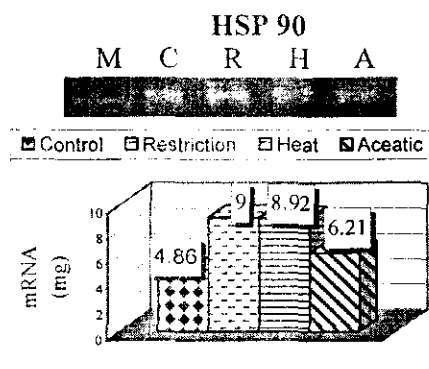


Figure 2: mRNA value of HSP 90 in jejunum as affected by feed restriction, heat exposure and acetic acid supply in NZW rabbit kits

Table 1: Activities (U/ml) of some enzymes of small intestine content in NZW rabbit at 1st week post weaning

Enzymes	Control	Restriction	Heat	Acetic	±SE
Amylase	0.76 ^c	1.13 ^b	1.34 ^{ab}	1.44 ^a	0.07
Protease	0.037 ^c	0.066 ^a	0.052 ^b	0.047 ^{bc}	0.004
Lipase	0.038 ^b	0.030 ^b	0.010 ^c	0.066 ^a	0.0031

^{a,b,c} Values having different superscripts within the same row are significantly different ($P < 0.05$).

Table 2: Blood picture in NZW rabbit kits at 1st week post weaning as affected by feed restriction, short heat exposure and acetic acid supplementation

Items	Control	Restriction	Heat	Acetic	±SE
Hemoglobin(g/100ml)	10.2	9.4	10.1	9.8	0.45
Hematocrit (%)	30.7	28.7	30.2	29.2	1.3
ErythrocytesX10 ⁶	3.9	3.6	3.7	3.6	0.14
Leukocytes X10 ³	6.2	6.8	5.5	5.8	0.85

Table 3: Differential count of leukocyte in NZW kits rabbit at 1st week post weaning as affected by feed restriction, short heat exposure and acetic acid supplement in NZW rabbit kits.

Items	Control	Restriction	Heat	Acetic	±SE
Neutrophils (N)	48.0 ^{ab}	44.5 ^c	46.7 ^b	49.5 ^a	0.55
Lymphocyte (L)	43.8 ^b	48.2 ^a	47.7 ^a	43.3 ^b	0.80
Monocyte	3.6	3.5	2.7	3.7	0.68
Esinophile	3.7	3.7	3.5	3.4	0.51
N/L ratio	1.10 ^a	0.92 ^b	1.00 ^b	1.15 ^a	0.027

^{a,b} Values having different superscripts within the same row are significantly different (P<0.05).

Table 4: Values of pH, absolute length and relative length for some organs of gastrointestinal tract as affected by feed restriction, heat exposure and acetic acid supplement in NZW rabbit kits.

Items	Control	Restriction	Heat	Acetic	±SE
Small intestine length (mm)	234.7 ^b	216.2 ^c	233.2 ^b	249.7 ^a	4.6
Small intestine relative length	0.29 ^a	0.23 ^b	0.26 ^{ab}	0.27 ^{ab}	0.016
Small intestine pH	6.8	6.7	6.8	6.8	0.059
Caecal pH	5.2	5.2	5.4	5.1	0.099
Villous height (µm)	433.2 ^b	487.5 ^a	489.7 ^a	435.5 ^b	12.4
Crypt depth (µm)	82.7 ^c	87.5 ^{ab}	90.0 ^a	86.7 ^b	0.97

^{a,b} Values having different superscripts in the same row are significantly different (P<0.05).

Table(5) : percentage of mortality during 1st, 2nd and 4th week post weaning as affected by feed restriction ,heat exposure and acetic acid supplement in NZW rabbit kits.

mortality%	Control	Restriction	Heat	Acetic
1 st wk post weaning	6.7	0.0	0.0	13.3
2 nd wk post weaning	13.3	0.0	6.7	13.3
4 th wk post Weaning	33.3	13.3	13.3	20.0

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الملخص العربي

تأثير التعريض الحراري القصير والتحديد الغذائي المتزن وإضافة حمض الخليك علي القناة الهضمية والنفوق بعد الفطام في الأرانب مع التأكيد علي بروتينات الصدمة الحرارية

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تهدف الدراسة إلي تقييم إثارة بروتينات الصدمة الحرارية ٧٠ و ٩٠ عن طريق التعريض الحراري القصير و التحديد الغذائي المتزن و إضافة حمض الخليك وذلك لحماية الأمعاء أثناء الفطام. استخدم في هذه الدراسة ٨٠ أرنب عمر ٣٠ يوم (فطام) وقد تم تقسيمهم إلي ٤ مجاميع كالتالي: المجموعة الأولى: مجموعة كنترول. المجموعة الثانية: تم معاملة الأرانب المفطومة بالتحديد الغذائي خلال الأسبوع الأول بعد الفطام (٧٥% من الأكل الحر). المجموعة الثالثة: تم تعريض الأرانب المفطومة لحرارة مرتفعة ($34 \pm 2^{\circ}\text{C}$) لمدة ساعة مرة واحدة. المجموعة الرابعة: تم إضافة ٠.٥ % حمض خليك في ماء الشرب خلال الأسبوع الأول بعد الفطام.

ويمكن تلخيص أهم النتائج كما يلي : لوحظ أن كمية ر. ن. أ. الرسول لعائلتي ٧٠ و ٩٠ من بروتينات الصدمة الحرارية في اللفانفي كانت في مجموعة التحديد الغذائي عالية مقارنة بالمجموعات الأخرى. كما أن كمية ر. ن. أ. الرسول لعائلة ٩٠ في اللفانفي في مجموعتي التعريض الحراري وحمض الخليك كانت أعلى من الكنترول. النشاط الإنزيمي لكل من الاميليز والبروتيز في محتويات الأمعاء في مجموعة التعريض كانت أعلى من الكنترول بينما كان الاميليز أقل. كما لوحظ انخفاض معنوي بين نسبة الخلايا المتعادلة /الخلايا الليمفاوية في كل من مجموعة التحديد الغذائي والتعريض الحراري والذي يعتبر دليل جيد علي زيادة المناعة الأولية. كان ارتفاع وعمق الخملات في منطقة اللفانفي في مجموعة الكنترول أقل مايمكن مقارنة بالمجموعات الأخرى.

ويمكن القول أن تطبيق التحديد الغذائي بعد الفطام مباشرة يؤدي إلي زيادة التعبير الجيني لبروتينات الصدمة الحرارية (كل من العائلة ٧٠ والعائلة ٩٠) والذي ينعكس علي حماية تركيب الخملة والوظيفة المناعية ضد بعض أشكال الإجهاد. كما أن التحديد الغذائي المتزن والتعريض الحراري القصير وإضافة حمض الخليك قلل من نسبة النفوق بعد الفطام بنسبة ١٣.٣ و ١٣.٣ و ٢٠% علي التوالي مقابل ٣٣.٣ في الكنترول.