Egyptian Journal of Rabbit Science, 24 (1): 19 - 34(2014)

EFFECT OF ACETIC ACID ADDITION ON GROWTH AND SOME PHYSIOLOGICAL AND IMMUNOLOGICAL CHARACTERISTICS OF GROWING RABBITS.

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This experiment was carried out to evaluate the effect of acetic acid addition on growth and some physiological and immunological characteristics of growing rabbits. A total of 36 New Zealand White (NZW) weaning male rabbits with average live body weight of 685 ± 25 g were randomly divided into four treatment groups (9 rabbits each). The 1st group received basal untreated pelleted diet and served as a control group, while the 2nd, 3rd and 4th groups fed the basal diet with 0.5, 1.0 % and 1.5% acetic acid, respectively.

The obtained results showed that *live body weight and feed intake did not significantly differed due to treatments. Mortality rate significantly (P<0.05) decreased with increasing acetic acid level than those of low level (0.5 %) and of the control group. Red blood cells (RBCs) count, hemoglobin (Hb %), packed cell volume (PCV, %), lymphocyte and heterocyte percentages of blood were significantly and white blood cells (WBCs) insignificantly differed due to acetic acid treatment. Blood plasma contents of total protein, albumin and globulin insignificantly increased and total lipids, triglycerides, totalcholesterol, uric acid and transaminase enzymes of liver (AST and ALT) were significantly (P<0.05) differed due to acetic acid treatment. Rabbits which supplemented with 1.5% of acetic acid were accompanied by the highest values of these traits than those of control group. The activities of digestive enzymes (amylase, lipase and protease) were significantly (P<0.05) increased in groups treated with different levels of acetic acid compared with control group. Number of colonies bacteria Escherichia coli and Salmonella were significantly (P<0.05) decreased in gut of rabbit groups due to acetic acid treatment compared with those of control group. Ileum and ceacum pH values were insignificantly differed due to treatments.*

The obtained results indicated that addition of acetic acid showed improvement in digestive enzyme activities, some blood

hemato-biochemical constituents, immunological traits and decreasing mortality rate of growing rabbits, especially in rabbits received 1.5% of acetic acid.

Key words: Acetic acid, blood picture, blood components, growth performance, rabbits.

Rabbit farming has significant potential and useful contribution in improving meat supply, food security and national economy in developing countries (Anjana and Goswami, 2012 and Ebeid *et al*., 2013). Although there is an increasing interest in enterprises of raising rabbits with different sizes in Egypt, several difficulties and constraints stand against the successfulness of these enterprises. Digestive diseases are currently the main cause of morbidity in growing rabbits, resulting in dramatic mortality rate increases in fattening rabbitries (Rosell *et al*., 2009). The activity of the digestive enzymes in pancreatic tissue is low after 5 days post-weaning due to interaction with out factors which may increased the risk of developing post-weaning diarrhea (Hedemann and Jensen 2004).

In livestock production system, antibiotics are commonly used to animals to prevent disease and metabolic disorders, as well as feed efficiency. However, in recent years, public concern over routine use of antibiotics, in livestock nutrition has increased due to the enhance of antibiotics resistant bacteria that may represent a **threat** to human health. Consequently, considerable effort has been devoted towards developing alternatives to antibiotics. Organic acids traditionally have been used as food preservatives. (Naidu, 2000) reported that these acids retard microbial growth and contribute desirable sensory properties to a number of foods. Acetic acid historically diluted in the form of vinegar, has been utilized perhaps longer than any other preservatives for its antimicrobial effect, which influences food keeping-quality, wholesomeness, and safety. Organic acids have inhibiting action on the intestinal bacteria competing with the host for available nutrients, so these acids are predictable replacement for antibiotics in rabbits diet (Hyden, 2000 and Falcao-e-Cunha *et al*., 2007). Organic acids used in feed industry regularly for contracts and their many benefits are now admitted, whereas, the antifungal property of organic acid and antibacterial activity against anaerobic opportunistic pathogens (Cherrington *et al.*, 1990; Skrivanova *et al.*, 2006). The increased in pressure on livestock industry to phase out the use of prophylactic dosages of antibacterial growth promoters in the European Union due to microbial resistance in animals and human and the potential to do some in other parts of world has stimulated increased interest in alternative natural growth promoters (Fature and Matanmi, 2008).

Therefore, the present study intent to investigate the effect of diet supplemented with acetic acid on growth performance, some blood constituents and gut bacteria count in growing rabbits.

MATERIALS AND METHODS

The present study was performed at rabbitry farm of the Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. A total number of 36 male New Zealand White (NZW) rabbits weaned at 35 days of age with average weight of 685 ± 25 g were randomly divided into four treatment groups (9 rabbits each) in completely simple randomized design. The 1st group was received untreated basal pelleted diet and served as a control group. Meanwhile, the 2nd, 3rd and 4th groups' fed on the same basal diet supplemented with 0.5%, 1% and 1.5% acetic acid, respectively. Rabbits were individually housed in galvanized wire cages (Dimensions of 45 × 35 × 40 cm) until the end of the experimental period. All rabbits were kept under the same managerial, hygienic and environmental conditions, fed ad-libitum and fresh water was automatically available all the time by stainless steel nipples fixed in each cage. All rabbit cages were equipped with feeders and nipples. The chemical composition of the basal diet was 18. % crude protein, 13.5% crude fiber, 2.31% ether extract and 2650 digestible energy (Kcal/kg). The diet was formulated according to NRC (1977).

Live body weight (LBW) was recorded individually for each rabbit at 2, 4 and 6 weeks of the experimental period. Daily feed consumption (DFS) was determined precisely and calculated as gram per rabbit per day, during the whole experimental periods. Mortality rate (MR, %) was recorded daily throughout the experimental period.

At the end of experimental period four mail of each group were weight, sacrificed for slaughtering. Blood samples were collected into 10 ml EDTA tubes, and then gently mixed. The erythrocyte (RBCs), the total leukocyte (WBCs) and the differential leukocyte counts, the packed cell volume (PCV) and hemoglobin (Hb) concentrations were determined as described by Ewuola and Egbunike (2008). Blood plasma samples were stored for further determination of total protein (TP), albumin (Alb), total lipids (TL), tri-glycerid (TG) and total cholesterol (TC). While, plasma globulin (Glb) was deduced as the difference between TP and Alb values (Fonseca *et al*., 2004). Uric acid (UA) was determined according to (Tietz, 1986), Alanine-aminotransferase (ALT) and Aspartate- aminotransferase (AST) were determined according to Reitman and Frankel (1957). Amylase activity was determined using the method of Somogyi (1960). Lipase activity was assayed using the method described by Tietz and Fiereck

(1966). Protease activity was analyzed using the method of Lynn and Clevette-Radford (1984). On slaughtering, gastrointestinal removed for collection of small intestine and caecum contents into tubes to determine pH values and bacteria number, pH of caecum and ileum using pH digital-pH meter. E. coli bacteria Inventory was carried out according to the method described by Cheney *et al.* (1979), while Salmonella count carried out according to the method of Giannella *et al.* (1973).

Statistical analysis

The obtained data were statistically analyzed in completely simple randomized design (Snedecor and Cochran, 1982) using SAS (1998).
Data was analyzed by adopting the following model:

$$Y_{ij} = U + T_i + e_{ij}$$

Y_{ij} = An observation, U = The overall mean, T_i = The effect due to treatment levels (i = 0.5, 1 and 1.5% acetic acid), e_{ij} = A random error.

Differences among means were tested by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance:

The obtained results of live LBW and DFS insignificantly affected by treatments throughout all the experimental periods (Table 1). Our finding agreement with those obtained by Amaefule *et al.* (2011) in growing rabbits and Kopecky *et al*. (2012) in broiler they showed that diets supplemented with acetic acid did not significantly altered growth performance traits. On the other hand, these results partially agree with those obtained by Radwan and Abdel-Khalek (2007) who explained that there were no significant differences in feed intake of all experimental growing NZW rabbits due to using 0.5% of acetic acid, while the final live total body weight significantly increased when used 0.5% acetic acid compared with control group. Mortality rate significantly ($P<0.05$) decreased linearly with increasing dietary acetic acid level. These results are in agreement with those obtained by Romero *et al.* (2010) and Romero *et al.* (2011) who reported a sharp reduction in the mortality rate due to diarrhoeal syndromes when rabbits were fed diets including short-chain organic acids. Skrivanova *et al.* (2006) stated that beyond the antifungal property of organic acids, some other benefits such as their antibacterial activity against anaerobic opportunistic pathogens and reduce mortality rate due to digestive diseases. Also, Cardinali *et al.*, 2007 observed that when rabbits feed organic acids drastically reducing the mortality percentage in a

context of high incidence of digestive diseases or after an experimental infection with harmful pathogenic bacteria.

Blood heamatology:

Data in Table 2 showed that WBCs count insignificant changes in rabbit groups treated with all various levels of acetic acid compared with these of the control group. However, rabbits treated with 1.5% acetic acid increased insignificantly than other groups and control group. Lymphocytes and heterocyte percentages significantly ($P<0.05$) increased in rabbit groups treated with different levels of acetic acid compared with control group. These results may be due to increase the activity of immune system. That's where, the response of gut-associated lymphoid tissue to the diet supplemented with organic acids in growing rabbit to be determined (Romero *et al.*, 2011). Since the appendix and Peyer's patches serve a major role in the synthesis of antibodies against intraluminal antigens (Dasso *et al.*, 2000). The count of RBCs, Hb % and PCV % significantly ($P<0.05$) decreased in rabbits group fed on diet supplemented with 1.5% acetic acid compared with the control or other treated groups (Table 2). These results are in agreement with those obtained by Askar (2012) who stated that WBCs number and the percentage of lymphocyte significantly increased in broiler treated by phenyl lactic acid. Abdel-Kafy *et al*. (2008) showed that RBCs count; hemoglobin concentrations and hematocrit percentage were decreased in growing NZW rabbits treated by acetic acid compared with control group. Wang *et al.* (2009) found in pigs that lymphocyte percentage linearly increased as dietary phenyl lactic acid increased and WBCs counts tend to increase as phenyl lactic acid level increase. The increase of WBCs number may be due to the activation of animals defence mechanisms and immune system (Abdel-Tawab and Abbassy. 2012). Wang *et al.* (2009) and Czerwinski *et al.* (2010) reviewed that organic acids could stimulate the immune system, it has been clearly established that several organic acids can affect microorganisms population of gut intestine that are necessary for gut immune system development.

Blood biochemical constituents:

The results of TP, Alb and Glb concentrations due to acetic acid supplementation insignificantly increased in rabbits group treated with 1.5% acetic acid compared with other groups and control group (Table 3). these findings are in agreement with those obtained by Radwan and Abdel-Khalek (2007) in growing rabbits, Wang *et al.* (2009) in pigs and Askar (2012) in quail they found that TP and Alb concentrations were higher when animals fed treated diet supplemented with acetic acid in rabbits or supplemented with phenyl lactic acid in (pigs and quail) compared with the values of the

control group, the authors reminded that the highest value of total protein accompanied by increasing acids level.

Total lipids significantly ($P<0.01$) increased in rabbit groups treated with different levels of acetic acid compared with the untreated group. While, TG and TC significantly ($P<0.01$) increased in rabbits treated by 1 and 1.5% of acetic acid only compared with the control group. While TG and TC concentrations were decreased with low level of acetic acid compared with untreated group. The results agree partially with those observed by Radwan and Abdel-Khalek (2007) who found that total lipid concentration increased in rabbit treated with acetic acid; however the author observed that cholesterol level significantly decreased compared with the untreated group. Meanwhile, our findings differ with the result revealed by Hassan *et al.* (2008) who showed that total lipids, triglycerides and cholesterol significantly decreased due to the treatment by acetic acid in laying hen. Sheikh *et al.* (2010) observed that blood contents of cholesterol did not significantly differ as results of treatment by some organic acids in broiler.

Markedly decrease in AST activity in groups treated with 0.5 and 1% of acetic acid, while the activity of AST significantly ($P<0.05$) increased in rabbits treated with 1.5% acetic acid compared with control group (Table 3). ALT activity was significantly ($P<0.01$) increased in rabbit groups treated by 1.0 and 1.5% of acetic acid compared with control group. While, uric acid significantly ($P<0.05$) decreased in all treated groups compared with control group. The activities of AST and ALT enzymes were generally decreased in rabbits group treated with low level of acetic acid (Table 3). The liver is responsible for detoxification process of metabolism and biosynthesis of energetic macromolecules for different essential functions (Aly *et al.*, 1997). Also, it is the first organ to face any foreign molecule that is carried through portal circulation and it is subjected to most damage. The increase of the indicated enzymes is reflecting the lesion in liver (El-Demerdash and Nasr, 2014). In addition, AST and ALT are important enzymes as specific indicators for liver function and in plying a role in amino acids catabolism and biosynthesis (Harper, 1979).

Result in (Table 4) revealed that digestive enzymes (amylase, lipase and protease) activities significantly ($P<0.01$) and linearly increased in rabbit groups treated with various levels of acetic acid compared with control group. Whereas rabbits group that received 1.5% of acetic acid showed the highest value of different digestive enzymes. These results may be due increase the synthesis of digestive enzymes consequence, lead to improved digestion. Whereas, Cardinali *et al.* (2007) explained that further benefits of organic acids that would stem from a direct stimulation of gastrointestinal mucosa growth. These results are in agreement with those observed by Abdel-Kafy *et al.* (2008) who found that activities of amylase

and protease enzymes in small intestine were higher of rabbit groups exposure for heat and acetic acid compared with control group. Organic acids in the popeline help to increasing digestibility of proteins and regulating the micro flora in the gut (Philipsen, 2006).

The number of colonies bacteria *Escherichia coli* and Salmonella were significantly ($P<0.05$) decreased in gut of rabbit groups which treated by acetic acid compared with those of the control group. Organic acids have anti-biotic like action, through inhibiting action of the intestinal bacteria (Hyden, 2000). Acidification of diets with organic acids is widely used to microbial degradation and improving productive performance of poultry and rabbits (Panda *et al*., 2006 and Radwan and Abdel-Khalek, 2007). Also' many investigators reviewed that chick fed on organic acids reduced E.coli lesion score as compared to those fed on the antimicrobial compounds (Waldroup *et al.*, 1995, Engberg *et al*., 2001 and Panda *et al*., 2006).

Data in Table (4) showed that ileum and cecum pH were insignificantly decreased in rabbit groups fed on diets supplemented with different levels of acetic acid when compared with unsupplemented group. Our results are in agreement with those obtained by El-Allawy (2001) who found that citric acid (5.0 g/ kg diet) had no significant effect on rabbit cecum pH values. Radwan and Abdel-Khalek (2007) observed that diets supplemented with organic acids (acetic acid and lactic acid) insignificantly increased volatile fatty acids production which reflected on reduce the pH values of cecum and ileum. Also, Romero *et al.* (2011) observed that caecal pH value was decreased with increasing caecal volatile fatty acids when rabbits fed diets supplemented with organic acids.

In conclusion, the current study proved that acetic acid addition to growing rabbit diets with level of 1.5% could be exerting some positive effects on some blood biochemical and immune constituents and rabbit performance.

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تأثير اضافة حمض الخليك على النمو و بعض الصفات الفسيولوجية والمناعية فى الارانب النامية

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اجريت هذه التجربة لدراسة تاثير اضافة حمض الخليك على اداء النمو وكذلك بعض الصفات الفسيولوجية والمناعية فى الارانب حيث تم استخدام عدد ٣٦ ارنب ذكر من سلالة الارانب النيوزيلندى فطمت عند ٣٥ يوم وكان متوسط وزن الارنب (٦٨٥ ± ٢٥) جرام. تم تقسيمهم عشوائيا الى اربع مجموعات (٩ ارانب بكل مجموعة). المجموعة الاولى غذيت على العليقة الاساسية واستخدمت كمجموعة مقارنة. والجموعة الثانية والثالثة والرابعة غذيت على العليقة الاساسية مضاف لها ٠,٥% و ١% و ١,٥% حمض الخليك على الترتيب. واوضحت النتائج المتحصل عليها ان وزن الجسم واستهلاك العلف لم يتاثر بصورة معنوية عند اضافة حمض الخليك بالمستويات المختلفة فى حين انخفضت نسبة النفوق فى المجموعات التى غذيت على علائق مضاف لها حمض الخليك عند مقارنتها بمجموعة المقارنة. عدد كريات الدم الحمراء وتركيز الهيموجلوبين وتركيز المكونات الخلوية تاثرت بصورة معنوية بالمعاملة بحمض الخليك حيث ان المجموعة التى تغذت على عليقة مضاف لها ١,٥% حمض الخليك اظهرت اقل قيم لهذه القياسات عن مجموعة المقارنة والمجموعة التى غذيت على ٠,٥% حمض الخليك اظهرت اعلى قيم لهذه القياسات.عدد كريات الدم البيضاء زادت بصورة غير معنوية للمجموعات التى تم معاملتها بحمض الخليك فى حين ان خلايا الدم البيضاء المختلطة والخلايا الليمفاوية زادت بصورة معنوية عن مجموعة المقارنة. محتوى البلازما من بروتينات الدم الكلية والالبيومين والجلوبيولين زادوا بصورة غير معنوية بينما الدهون الكلية والجليسريدات الثلاثية والكوليستيرول ارتفعوا بصورة معنوية فى

بلازما دم المجموعات التى عوملت بحمض الخليك عن مجموعة المقارنة وكانت اعلى قيم لهذه القياسات سجلت فى المجموعة التى عوملت ١,٥% حمض الخليك بينما المجموعة التى احتوت ٠,٥ % انخفض فيها مستوى هذه القياسات بصورة معنوية عن مجموعة المقارنة. حمض اليوريك انخفض بصورة معنوية فى كل المجموعات التى تم معاملتها بحمض الخليك عن مجموعة المقارنة. نشاط انزيمات الاميليز والليبيز والبروتييز زاد بصورة معنوية عن مجموعة المقارنة فى كل المجموعات التى تم معاملتها بحمض الخليك فى حين انخفضت درجة الحموضة فى الامعاء والقولون بصورة غير معنوية فى المجموعات التى تم معاملتها بحمض الخليك عن مجموعة المقارنة.

التوصية: أوضحت النتائج المتحصل أن المعاملة بحمض الخليك ادى الى تحسن فى نشاط الانزيمات الهاضمة وبعض قياسات الدم الهيماتولوجية والبيوكيميائية وبعض القياسات المناعية للارانب النامية وانخفاض نسبة النفوق خاصة فى المجموعة المعاملة بتركيز ١,٥% حمض الخليك.