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INFLUENCE OF 4SUPPLEMENTAL ACIDFIERS ON PHYSIOLOGICAL AND PRODUCTIVE PERFORMANCE IN WEANING RABBITS

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ABSTRACT

The purpose of the present study was to determine if individual organic acids (Lactic(L), Acetic(A) and Citric (C) or mixture organic acids (LA, LC, AC and LAC) supplementation to drinking water would have a beneficial effect on physiological and productive performance of weaning rabbits. Seventy two weaned New-Zealand White rabbits (NZW) at 6 week of age weighing an average of 660 g were divided into eight groups (9 rabbits each), Rabbits of group (G1) received plain water. Rabbits of group 2,3,4 received water supplemented with 0.5 ml/ Litter (L), (A) or (C). Whereas, Rabbits of group 5,6,7 and 8 received water supplemented with 0.5 ml/Litter a total of a combination of(L+A), (L+C), (A+C) and (L+A+C). The rabbit houses were made of available local materials (mud, wood etc...,). Rabbits were fed ad libitum on pelleted ration contain 17.0% Crude protein ,2530 kcal/kg Metabolizable energy (ME), 12.4% Crude fiber. The study was continued during fattening period, from 6-12 weeks of age, from April to June 2004.

The results showed that lactic, acetic or citric acids separately had no significant effect on hematocrit values and hemoglobin concentrations. However, red blood cells counts and white blood cells counts increased significantly compared to the control groups. significant reduction was observed in blood pH, cecum pH and E.Coli count compared to the controls. The total anaerobic bacteria decreased significantly in G4 but no significant decrease was observed in G2 and G3. Marketing body weight and body weight gain increased significantly compared to the controls. However, feed conversion improved in the G2 and G4 only. Feed intake decreased in G2 only.

Results indicated that groups received mixtures of organic acids (L+A), (L+C), (A+C) and (L+A+C) had slightly increased hematocrit in G5, G7 and G8 compared to the control. Hemoglobin, red blood cells counts and white blood cells counts were increased significantly in all combination groups. Significant reductions were observed in blood pH, cecum pH, total anaerobic bacteria and E. coli counts in all combination groups. Groups received combinations of organic acids in drinking water had significantly increased marketing body weight and body weight gain, except G6, compared to the controls. Feed conversion increased in G5 and G6 compared to the control. Feed intake increased in all combination treated groups.

It was concluded that the significant use of citric acid significant or the use combination of organic acids (L+A), (A+C) and (L+A+C) in drinking water of weanling rabbits had beneficial effects throughout the growing period and improved hematological parameters, cecum microflora and productive traits.

Keywords : Rabbits, organic acids, entireties, E. coli . productive performance.

INTRODUCTION

The gastrointestinal tract (GIT) is a very complicated organ and is the obliged passage of the nutrients that support basal metabolism, growth and maintenance, supplying the resources to support the immune, skeletal and

nervous systems (Ferket, 2000). The pathogenic intestinal bacteria can cause diarrhea, infection, liver malfunction, and a reduction in digestion and absorption of nutrients. The beneficial bacteria can inhibit the growth of pathogenic bacteria by diverse mechanisms, stimulate the immune system, synthesize vitamins,... etc.(Macari, 2001).

Growing rabbits are sensitive to enteric diseases and especially when they are exposed to negative impacts, e.g. weaning or heat stress, causing high economic losses. This problem can be avoided by using antibiotics. However, the antibiotics as growth promoter have been under scrutiny for many years and have been removed from the market in several countries. Their usefulness has seldom been contested, it is their relatedness with similar antibiotics used in human medicine and the possibility that their use may contribute to the pool of antibiotic resistant bacteria that causes concerns. (Mroz, *et al.*, 2001 and Fortun and Boullier, 2004). A very important objective of dietary acidification is the inhibition of intestinal bacteria competing with the host for available nutrients, and a reduction of possibly toxic bacterial metabolites, such as ammonia and amines, that leads to weight gain improving of the host animals. Thomlinson and Lawrence (1981) observed a lower gastric pH when 1% lactic acid was added to drinking water offered to gastric cannulated piglets. Furthermore, lactic acid delayed the multiplication of an enterotoxigenic E. coli and reduced the mortality rate of the animals. The supplementation of milk with 1% lactic acid resulted in lower counts of coliform bacteria and lactobacilli in the stomach and duodenum of 2 weeks-old weaned pigs as compared to normal milk. Further investigators studied the influence of supplementing acidifiers on blood constituents in weanling rabbits.

The present study aimed to investigate the effect of lactic, acetic and citric acids supplementation in drinking water (separately or in a mixture) on some productive traits, blood constituents, pathogenic microorganisms and immune system in rabbits from weaning up to marketing age.

MATERIALS AND METHODS

This study was carried out at a private rabbitry in north east Dakahlia Governorate from April to June 2004. Seventy two weaned New Zealand White rabbits (NZW) at 6 weeks of age weighting about 660g on the average were used in this study. Rabbits were kept in groups (2 rabbits). They were randomly divided into eight groups, nine animals in each. The 1st group received plain water and severed as control group, while the other seven groups received water supplemented with different acidifiers (Table 1).

About 3 ml blood, were collected from the experimental animals (5 rabbits/group) at 14 weeks of age from the marginal ear vein in heparinized test tubes. Blood samples were taken to determine hematocrit values (Ht %), hemoglobin concentration (Hb g/dl), red blood cells (RBCs), white blood cells (WBCs) and blood pH using pH paper in fresh blood as described by (Feldman *et al.*.,2000).

Samples of cecum content were taken individually from five animals in each group after slaughter at the end of the experiment. Cecum fluid

content was obtained, after slaughter, to count total anaerobic bacteria and E.Coli in their selective media as described by Collins *et al.*.(1995). In addition, cecum pH was measured by using pH meter in filtrate cecum content. Statistical analysis was conducted using one way analysis of variance (SAS, 1999). Duncuan test (Duncan, 1955) was used to detect significant differences among means of the experimental groups.

Table (1): supplemented organic acids as treated groups

Group	Acids	Supplementation rate (ml/Litre)
1	control	Plain water
2	Lactic	0.5 ml/liter
3	Acetic	0.5ml/liter
4	Citric	0.5ml/liter
5	Lactic + Acetic	0.25 ml of each /liter
6	Lactic + Citric	0.25 ml of each /liter
7	Acetic + Citric	0.25 ml of each /liter
8	Lactic +Acetic + Citric	0.17 ml of each /liter

Rabbits were fed ad libitum on pelleted diet contained 17.0% crude protein, 2530 kcal ME/kg and 12.4% crude fiber. Fresh water was available at all times and supplemented daily with the different acids.

RESULTS AND DISCUSSION

1-Hematological responses:-

Hematological data for growing NZW rabbits as affected by organic acids administration from 6-14 weeks of age are illustrated in Table 2. The results showed that there were no changes in hematocrit values and hemoglobin concentrations in groups received the separate organic acids (lactic, acetic or citric) compared to the control. However, Red blood cells count increased significantly in the groups that received the separate organic acids compared to the controls. Groups received mixtures of organic acids (LA, LC, AC and LAC), in drinking water, had significantly increased ($P<0.05$) on hematocrit (Ht), hemoglobin concentration (Hb) and red blood cells count (RBCs) in most all combination groups compared to the controls. These increase in hematocrit, hemoglobin and red blood cells count values may be due to increasing the activity of metabolic cycle (citric acid cycle for example) and the improved growth performance as a result of lactic, acetic and citric acids supplementation in a mixture form (El-Kholy, 2003). These results are in full agreement with El-Kholy (2003) who found that hematocrit, hemoglobin and red blood cells increased by using synertox (a commercial product contain lactic, acetic and citric acids). Moreover, Ismail. *et al.*,2002 found that hematocrit, hemoglobin and red blood cells increased by using acitrol (a commercial product contain lactic, acetic and citric acids). However, The blood pH decreased significantly in the treated groups compared to the control group. The significant reduction in blood pH due to the organic acids treatments are in agreement with Ismail. *et al.*, 2002. They found that blood pH decreased in NZW rabbits when they received acitrol.

Table (2): Least square means of hematocrit value (Ht,%) , hemoglobin concentration(Hb, g/dl) , blood PH, total red blood cells (RBCs, $10^6/mm^3$) and total white blood cells ($10^3/mm^3$)and their standard errors (S.E.) of NZW rabbits as affected by some organic acids treatments from weaning to marketing.

Group	Treatments**	Ht	Hb	Blood PH	RBCs	WBCs
G1	Control	30.8^{c}	10.8^{d}	7.56^{a}	4.57^{b}	5.06^{d}
G2	Lactic(L)	32.0^{bc}	11.0^{d}	7.36^{b}	4.88^{a}	6.26^{abc}
G3	Acetic(A)	32.0^{bc}	11.0^{d}	7.26^{b}	4.77^{a}	5.78^{c}
G4	Citric(C)	32.0^{bc}	11.8^{dc}	7.26^{b}	4.76^{a}	6.72^{a}
G5	L+A	34.2^{ab}	12.9^{abc}	7.34^{b}	4.90^{a}	6.26^{abc}
G6	L+C	33.0^{abc}	12.4^{bc}	7.32^{b}	4.80^{a}	5.96^{bc}
G7	A+C	35.4^{a}	13.3^{ab}	7.26^{b}	4.91^{a}	6.40^{ab}
G8	L+A+C	35.4^{a}	13.7^{a}	7.34^{b}	4.94^{a}	6.10^{bc}
	S.E.	0.8	0.41	0.048	0.048	0.87
	Overall means	33.1±0.4	12.1±0.2	7.34±0.02	4.82±0.03	6.1±0.46

*Means, within trait, between treatments, with different superscripts, differ significantly (P≤0.05) from each other.

**The doses used were a total of 0.5 ml/liter drinking water of each acid or equal parts of each organic acid.

2-Immune response:

Data of total white blood cells (WBCs) and differential counts for growing NZW rabbits as affected by organic acids supplementation from 6-14 weeks of age are shown in Tables 2&3. Results indicated that the supplementation of the organic acids, in drinking water, lead to significantly increased white blood cells count (WBCs) in all of the treatments when compared to the controls.

Table(3): Least squares means of differential counts of white blood cells and their standard errors (S.E.) of NZW rabbits as affected by some organic acids treatments from weaning to marketing (6-14 weeks of age).

Group	Treatments**	Differential count of White blood cells					
		Lymphocytes	Neutrophils	N/L ratio	Monocytes	Basophils	Eosinophils
G1	Control	57.0^{b}	36.4^{a}	0.64^{a}	3.4^{ab}	1.6^{a}	1.4^{b}
G2	Lactic(L)	55.4^{b}	36.4^{a}	0.66^{a}	4.0^{a}	1.4^{a}	1.4^{b}
G3	Acetic(A)	56.8^{b}	37.4^{a}	0.66^{a}	3.2^{bc}	1.6^{a}	1.0^{b}
G4	Citric(C)	56.6^{b}	35.6^{a}	0.63^{a}	3.6^{ab}	1.4^{a}	1.4^{b}
G5	L+A	65.0^{a}	29.4^{b}	0.45^{b}	2.6^{dc}	1.6^{a}	1.2^{b}
G6	L+C	62.8^{a}	31.0^{b}	0.50^{b}	2.6^{dc}	1.4^{a}	1.8^{ab}
G7	A+C	64.2^{a}	30.0^{b}	0.47^{b}	2.4^{d}	1.6^{a}	1.8^{ab}
G8	L+A+C	63.8^{a}	29.2^{b}	0.46^{b}	2.6^{dc}	2.0^{a}	2.4^{a}
	S.E.	0.74	0.87	0.02	0.25	0.36	0.27
	Overall means	60.20 ±0.66	33.18 ±0.60	0.56 ±0.02	3.05 ±0.12	1.58 ±0.12	1.55 ±0.12

* Means, within trait, between treatments, with different superscripts, differ significantly (P≤0.05) from each other.

**The doses used were a total of 0.5 ml/liter drinking water of each acid or equal parts of each organic acid.

Lymphocyte percentage increased significantly in all the combination treated groups, compared to the control and group received separate organic acids. Neutrophils percentage and Neutrophils/ lymphocytes ratio (N/L) decreased significantly in all the combination treated groups(G5, G6,G7 and G8) compared to the control and groups received separate organic acids. Monocytes percentage also decreased significantly in all the combination treated groups compared to the control. There were no significant differences in basophils percentage due to the treatment. Eosinophils increased significantly in the combination of three acids group compared to the control group. It was clearly noted that, adding any organic acids, or their combination to the drinking water, of growing rabbits, lead to improving the immune response (increase WBCs, lymphocytes).These results are in agreement with El-Kholy (2003) who found that WBCs increased by using synertox . Also Ismail. *et al.*, 2002 reported that WBCs increase by using acitrol. The decreased of N/L ratio may be an indictor to a reduction of stress by adding different combinations of organic acids.

3-Microbial content of rabbits cecum:

3-1. Total anaerobic bacteria count and E.Coli :

Total count of anaerobic bacteria and E.Coli in the cecum are presented in Table 4. There was a significant ($P<0.05$) decrease in the number of total anaerobic bacteria in G4, G5, G6, G7 and G8 compared to the control. These decreases may be due to the increase in acidity of the hindgut contents as a result of the organic acids supplementation. The lowest value were obtained in the combination of three acids followed by the combination of two acids. E. Coli decreased significantly in all treated group compared to the control. The lowest number were also obtained in the 3-way combination followed by the 2-way combinations. These results are in agreement with Fuller (1989). He concluded that, lactic acids bacteria increased organic acids such as acetic and lactic which caused an inhibitory effect on the growth of other bacteria, especially pathogenic gram-negative bacteria.

Table(4): Least square means of cecum pH, total number of anaerobic bacteria (total count $x10^5$) and Escherichia coli (E.coli x 10^2) and their standard errors (S.E) of NZW rabbit as affected by some organic acids treatments from weaning to marketing.

Group	Treatment	Cecum pH	Total anaerobic bacteria	E.coli
	Control	6.4^{a}	6.5^{a}	739.6^{a}
G1	Lactic(L)	6.1^{b}	6.3^{ab}	667.8^{c}
G2	Acetic(A)	6.1^{b}	6.5^{a}	694.9^{b}
G3	Citric(C)	5.8^{d}	6.0^{bc}	622.7^{d}
G4	L+A	5.9^{c}	5.7^{c}	606.2^{d}
G5	L+C	5.8^{cd}	5.8^{c}	609.2^{d}
G6	A+C	5.9^{c}	5.2^{d}	556.4^{e}
G7	L+A+C	5.9^{c}	4.9^{d}	551.0^{e}
G8	S.E.	0.05	0.1	8.9
	Overall means	6.0±0.04	5.9±0.1	631±10.3

*Means, within trait, between treatments, with different superscripts, differ significantly ($P\leq0.05$) from each other.

**The doses used were a total of 0.5 ml/liter drinking water of each acid or equal parts of each organic acid.

Also, Kershaw *et al..*, (1996) reported that the addition of 1% lactic acid to the drinking water of pigs reduce the E.coli in the duodenum and jejunum.

3-2.Cecum pH:

Data in Table (4) showed that cecum pH decreased significantly in all treatments compared to the control group. The lowest value in this study were obtained in G4 and G5. This may have lead to reducing pathogenic bacteria, and simultaneously increasing the beneficial bacteria. These results are in agreement with El-Allawy (2001). She indicated that, acidifiers (organic acids: citric, fumaric, acetic and propionic acids and a commercial product: Acid-pak 4-way) supplemented the limited acids production in the stomach of young animals. This lead to lowering the gastric pH to an adequate level.

4-Productive trait:

Initial body weight (IBW), marketing body weight (MBW), body weight gain, Feed conversion and Feed intake from weaning (6 weeks old) until 14 weeks of age for growing NZW rabbits as affected by lactic, acetic and citric acids supplementations separately or in mixtures are presented in Table 5. There were no significant differences in the Initial Body weight of all group. By the end of the experiment the marketing body weight and body weight gain of most treated groups were significantly ($P<0.05$) heavier than the control (except G6). These results are in agreement with those of (El-kerdawy *et al.*, (1996); Abdel- Azeem *et al.*, (2000); El-Allawy, (2001). Feed intakes were higher in all treated groups compared to the control (except G2). Feed conversion were better in G2 and G4 compared to the controls. In the combination treated groups (G5 and G6) feed conversion was worse than the controls. The best feed conversion was that of the lactic acid treated group. These results are agreement with those of EL-Kerdawy, (1996);Abdel-Azeem *et al.*, (2000). Mortality rate was 2.77 % for the control group and 0 % for all the supplemented groups.

Table(5): Least square means of initial body weight (IBW, g), marketing body weight (MBW, g) and body weight gain (BWG, g) ± standard error of NZW rabbits as affected by some organic acids treatments from weaning to marketing and feed intake (FI, Kg/rabbit) and Feed conversion (FC, Kg feed/ Kg weight gain).

Group	Treatment**	IBW±E	MBW±S.E	BWG±S.E	FI	FC
G1	Control	660^{a} ±3.8	1671^{c}± 19	1009^{c} ±21	4.6	4.70
G2	Lactic(L)	658^{a}±3.8	1854^{a}± 17	1196^{a}±18	4.4	4.60
G3	Acetic(A)	658^{a}±3.8	1838^{a}± 17	1179^{ab}±18	5.3	4.95
G4	Citric(Ci)	660^{a}±3.8	1878^{a}± 17	1218^{a}±18	4.9	4.00
G5	L+A	653^{a}±3.8	1787^{b}± 17	1133^{b}±18	5.7	4.97
G6	L+Ci	663^{a}±3.8	1702^{c}± 17	1039^{c}±18	5.4	5.20
G7	A+Ci	661^{a}±3.8	1859^{a}± 17	1198^{a}±18	5.4	4.50
G8	L+A+Ci	653^{a}±3.8	1844^{a}± 17	1191^{a}±18	5.4	4.50
	Overall means	659±1.32	1808±10	1149.21±10.34	5.1	4.7

*Means within age, between treatments, with different lower case superscripts differ significantly ($P\leq0.05$) from each other.

**The doses used were 0.5 ml/liter drinking water of each acid or combination.

It may be concluded that, organic acids supplementations (especially citric acid) improved body weight and weight gain more efficiently than the other treatment groups. Organic acids supplementation decreased mortality in all treated groups during the post-weaning period. It may be lead to improved survival rate, in growing rabbits, with organic acids supplementation. These result are agreement with those of Abdel-Azeem, *et al.*, (2000); El-Allawy, (2001); Ismail *et al.*, (2002). Improvement of feed conversion, as a result of the addition of lactic and citric acids in drinking water, could be attributed to the reduction in feed intake with significant increases in live body weights and body weight gains.

CONCLUSION

It could be concluded from the present study, that the weaning rabbits could benefit by using lactic, acetic and citric acids separately or mixtures in drinking water. There organic acids act as growth promoting factor, by improving the immune system. They reduce pathogenic microorganisms simultaneously increase the beneficial bacteria, which reflect on improving the digestive system and lead to more gain.

REFERENCES

Abdel-Azeem,F., Y.M,EL-Hommosany and Nematallah,G.M .Ali., (2000). Effect of citric acuds in diets with different starch and fiber levels in oroductive performance and some physiological traits of growing rabbits. Egyptian Journal of Rabbit Science, 10: (1): 121-145.

Collins,C.H, P.M. Lyne and J.M. Grange (1995). Collins and Lyne's microbiololgical methods. Butterworth Heinemann Ltd, Oxford.

Duncan, D. B, (1955). Multiple range and multiple F test. Biometrics, 11:1-42.

El-Allawy, M. H. Hewida, (2001). The use of Bio-additive to improve rabbit performance. Ph,D,Thesis,Fac. Agri., Cairo, Egypt.

El-Kerdawy, D.M.A,(1996). Acidified feeds for growing rabbits. Egyptian Journal of RabbitScience, 6: (2): 143-156.

El-Kholy, K.H.M ,(2003).Amelioration of heat stress on rabbits. ph,D, Thesis, Fac. Agri., Cairo, Egypt.

Feldman, B.F., J.G.Zinkl and N.C. Jain, (2000). Schalm's veterinary Hematology. Lippincott Williams and wilkins, Philadeliphia, USA.

Ferket P.,(2000) Practical nutritional perspective on gut health and development. Proceedings 27th Annual Carolina Poultry Nutrition Conference and Soybean Meal Symposium, Nov. 15-16, Triangle Park, NC

Fortun-L. and Boullier (2004) Interactions between gut microflora and digestive mucosal immunity, and strategies to improve digestive health in young rabbits (1035:1067).

Fuller,R.,(1989). Probiotics in man and animals : A review. J. Appl.Bact., 66: 365- 378.

Ismail, A.M., M.A.Abou-El-Ella and A.A.Sedki,(2002). Blood metabolities, growth and reproductive performance and immune responsiveness in growing and doe rabbits as affected by acitrol treatments. Egyptian J. Agric. Res., 80:1789-1808.

Kershaw,G.F.,J.R.Luscombe, and D.J.A.Coli.,(1966). Lactic acid and sodium acrylate: Effect on growth rate and bacterial flora in the intestines of weaner pigs. Vet.Rec. 79:296-297.

Macari, M. A., (2001). Maiorka. Aspectos fisiológicos da qualidade instestinal pré- e pós- eclosão e produtividade em frangos de corte. XXII Seminário Avícola Internacional. Amevea. Colombia.pp.46-57.

Mroz, Z., A. W. Jongbloed, R. Von Der Weij-Jongbloed,and M.Øverland,(2001).Effects of adding potassium diformate and phytase excess for weaned piglet. In: Digestive physiology of pigs, Ed. by Lindberg J E, Ogle B, CABI publishing, p 305-307.

SAS, (1999). SAS User's Guide : Statistical Analysis System Institute, inc., Cary, N. C.

Thomlinson, J. R. and T. L. J. Lawrence,(1981).Dietary manipulation of gastric pH in the profilaxis of enteric disease in weaned pigs. Some field observations. Veterinary Record, 109:120- 122.

تأثير إضافة الأحماض العضوية علي الكفاءة الفسيولوجية و الإنتاجية في الأرانب النيوزيلندية المفطومة.

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أجريت التجربة علي الأرانب النيوزيلندي المفطومة لتحديد تأثير الأحماض العضوية علي مكونات الدم ومدي الاستجابة الوظيفية للجهاز المناعي و الميكروبات الضارة التي تنمو في الأعور والكفاءة الإنتاجية.

تم استخدام ٧٢ أرنب من النوع النيوزيلندي ابيض عمر٦ أسابيع ومتوسط وزن ٦٦٠ جم قسمت علي ٨ مجاميع تجريبية متساوية العدد تم إضافة ٠.٥ مللي علي لتر ماء الشرب من توليفات مختلفة للأحماض (اللاكتيك) و(الستريك) و(الاستيك) و(اللاكتيك+الاستيك)، (اللاكتيك+الستريك) و (الاستيك+الستريك) و (اللاكتيك+الاستيك+الستريك)

أظهرت النتائج أن إضافة الأحماض العضوية (اللاكتيك،الاستيك و الستريك) أدي إلى :

١-تحسنت قيم الهيماتوكريت، الهيموجلوبين و زادت عدد كرات الدم الحمراء .

٢-زادت خلايا كرات الدم البيضاء الكلية والليمفوثايتس وانخفضت نسبة النيتروفيل علي الليمفوثاليتس.

٣-انخفاض درجة الحموضة(PH) للدم والأعور.

٤- انخفاض عدد الميكروبات الضارة بالنسبة للأرانب التي تم إضافة الأحماض إلى مياه الشرب لها.

٥- زيادة الوزن الحي والوزن المكتسب وتحسن كفاءة التحويل الغذائي.

من هنا يمكن القول أن إضافة الأحماض العضوية أدى إلى رفع كفاءة الجهاز المناعي وانخفضت عدد الميكروبات الضارة في نفس الوقت زادت عدد الميكروبات النافعة للجهاز الهضمي كما تحسنت صفات الدم وزاد الوزن المكتسب وتحسنت كفاءة التحويل الغذائي وانخفضت معدلات النفوق نتيجة إضافة الأحماض العضوية. ونجد أن إضافة الأحماض بصورة خليط أعطت نتائج اعلي من الصور الفردية مما يدل علي وجود صفة تعاون بينهما.