Research Article

The Effect of Adding Malic and Acetic Acids to Drinking Water on The Productive Performance of Quail

Ali A. M. Al-Kuhla[1*], Yaman S. Fadhil[1], Saffanah K. Mahmood[2]

[1]University of Mosul /College of Veterinary Medicine/Department of Veterinary Public Health; [2]University of Mosul / College of Veterinary Medicine / Department of Anatomy.

Abstract | One hundred and twenty local male quails were used to investigate the effects of individual and combined malic and acetic acid in drinking water on the production performance and intestinal histomorphometry of quails. The quails were divided into 4 groups with 3 replicates for each group, 10 birds per each replicate. 1st group represented the control group, which was free of any additives. In 2nd and 3rd groups, malic and acetic acid respectively were added in drinking water with a percentage of 0.025%, while in 4th group both malic and acetic acids were added to drinking water with a percentage of 0.025% for each of them. During the experiment, the productive characteristics were studied including the live body weight, the rate of weight gain, the consumption and conversion of feed. Finally, all the bird were slaughtered for the purpose of histological examination. Tissue sections were obtained from the small intestine, then the villi length, width, surface area of villi, and the crypt depth were measured. Results demonstrated a significant ($P<0.05$) increase in the body weight of the birds in response to the dietary malic and acetic acids, while there were no significant differences in the rate of weight gain, the amount of feed consumed, and the feed conversion ratio. Histologically, it was observed that there was a significant ($P<0.01$) increase in the villi length, villi width, crypt depth and surface area in organic acid supplemented groups as compared to the control group. In conclusion, supplementation of organic acids (acetic and malic acid) is beneficial for quail production as it has better effects on intestine. Moreover, mixture of acetic and malic acid could be more helpful in digestion and absorption of nutrients in the small intestine.

Keywords | Acetic acid, Malic acid, Drinking water, Intestine, Quail

Received | March 09, 2023; **Accepted** | April 15, 2023; **Published** | June 25, 2023
***Correspondence** | Ali A M Al-Kuhla, University of Mosul /College of Veterinary Medicine/Department of Veterinary Public Health; **Email:** aliaug1976@uomosul.edu.iq
Citation | Al-Kuhla AAM, Fadhil YS, Mahmood SK (2023). The effect of adding malic and acetic acids to drinking water on the productive performance of quail. J. Anim. Health Prod. 11(3): 250-257.
DOI | http://dx.doi.org/10.17582/journal.jahp/2023/11.3.250.257
ISSN | 2308-2801

INTRODUCTION

It was observed that there was a remarkable increase in the growth performance of poultry birds when organic acids and organic compounds were used as alternatives to antibiotics (Abbas et al., 2022; Jovank et al., 2008). Adding these acids to poultry feed is important, however, these were also found beneficial to reduce the bacterial contamination of meat (Mulla et al., 2022) and salmonella infestation of birds which is a common cause of deterioration of intestinal walls that decreases the number of intestinal villi and reduces its length, thus inflicting a damage to the absorption of food compounds (Pelicano et al., 2005).

Several studies have been conducted on adding organic acids to poultry diets because of their advantage in lowering the pH of bacteria in the gastrointestinal tract as the organic acid infiltrate to the bacterial wall and thus inhibiting its natural physiological functions (Gauthier 2002). Organic acids, whether hydrolyzed or not, can penetrate the fatty part of the bacterial wall because it is lipophilic and then ionized inside the bacterial cell cytoplasm (Davidson , 2001). The low pH inside the bacterial cell lead to the inhibition of the action of many bacterial enzymes thus causes a large difference between the inside and outside pH of the bacterial cell wall. This, in turn, stimulate the bacterial cell to perform a special function called H-ATPase pump, which keeps pH inside the bacterial cell within the normal limits. This technique requires high energy that, eventually, leads to bacterial stress and stops its growth and reproduction and then causes death (Davidson, 2001).

The study of Moharrey and Mahzonich (2005) is amongst the studies that were conducted in this field. Malic acid was added with percentages of 0.5, 0.1, and 0.15 to the drinking water of broilers; a 0.5% supplementation exhibited a significant rise in the live body weight in comparison with the control group. Additionally, the weight gain rate is considered a clear indication of the productive process. As the researchers Frankenbach et al. (2001) noticed that when citric acid was added with percentages of 0, 2, 4, and 6 to the ration of broiler, there was a statistically significant rise ($p \le 0.05$) in the weight gain rate of the groups to which citric acid was added at the rate of 4, and 6% compared to the other groups.

Gheisari et al. (2007) supplemented the protected organic acids with a percentage of 0.2% and observed that it could improve the reproduction of beneficial microbes and reduce the harmful microflora found in the guts of poultry. The length of villi in cobb–500 and Ross–308 broiler breeds was observed higher in the duodenum, jejunum and ileum parts when acetic acid was added with concentrations of 1 and 2% in the diet (Mustafa et. al., 2004). Likewise, Attia et al. (2013) studied the feed prepared using various concentrations of acetic acid on the growth and production of the Japanese quail. They noticed that acetic acid had certain stimulatory effects on the bodys' immune system, which were represented by an increase in the cellular interactions in the intestine, in addition to the lymph nodes in the spleen tissue.

The excessive caution towards the severe problems in poultry is associated with the use of antibiotics that encourages the researchers to make more efforts to use food additives through the alternative strategic plans that include using the organic acids, their salts, probiotics, prebiotics, enzymes as well as their herbal extracts (Bauermann, 2006; Lim et al., 2023). Therefore, the purpose of this work was to investigate the impact of adding acetic and malic acid to the drinking water and the extent to which this addition affects the production characteristics and histomorphometry of small intestine in the local quail.

MATERIALS AND METHODS

One hundred and twenty of local male quails were used in an experiment for the period from 1 / 7 / 2022 until 5 / 8 / 2022. The management of animals in this research was tracked the strategies of the Institutional Animal Care and the Research Ethics Committee of the College of Veterinary Medicine, University of Mosul, with an ethical endorsement number of (UM.VET.2022.042). The total number of quails was divided into 4 groups and three replicates for each group, 10 birds per a replicate.

To conduct this research, a clean and sterilized poultry hall with dimensions of (5×20 meters) was used at the College of Veterinary Medicine / University of Mosul. The preventive health program for birds was applied by giving the birds the necessary vaccines. The starter ration was used for the first three weeks of the birds' life. After that, the finisher's ration was used in the last two weeks of the life of the birds and the feed was introduced manually and freely (ad-libitum). The drinking water was provided to the birds using plastic fountains and lighting was set continuously (23 hours) with 1 hour of darkness per a day during the experiment period. The necessary air-conditioning procedures were implemented by means of using electrical air-coolers that were used in the poultry hall and three mercury thermometers were used and they were put at the beginning, middle and end of the hall in an elevation that equals the bird's height. Before the birds were put in the hall, the hall was washed with water and detergents needed to clean and remove dust and impurities and then it was left for one day to dry. In addition to that, the places were sterilized by spraying them with a mixture of water and iodine to get rid of bacteria and parasites in case that they exist and leaving them for three days to dry. In addition to that all feeders and manual fountain were washed and sterilized. Birds were distributed to four groups, namely; the first one represented the control group and it is free from any additives, the second group to which malic acid ($C_4H_6O_5$; Scharlau, Spain) was added in the drinking water with a ratio of 0.25 gram/ liter (0.025%, w/v). The third group was supplemented with acetic acid (CH_3COOH; Scharlau, Spain) via adding in the the drinking water at the rate of 0.25 ml/liter (0.025%, v/v). While the fourth group was given both malic and acetic acid by adding in the drinking water of birds with the ratio of 0.025% of each of the acids. During the period of the experiment, the productive characteristics of the growth performance were studied, including body weight, weight gain rate, feed consumed and feed conversion ratio. The body weight was calculated weekly for all birds of each replicate using a sensitive scale (with a sensitivity of 0.00 g). The rate of weight gain was calculated by subtraction i.e., the living body weight in a given week minus the living body weight in the preceding week.

The amount of feed consumed was calculated depending on the amount of feed provided at the beginning of the week minus the amount of feed remaining at the end of the week. The feed conversion was determined by dividing the amount of the feed consumed by the average body weight every week. When the experiment was over, all the birds were slaughtered, tissue sections were taken from the small intestine and after that the villi length, width, surface area of villi, and the depth of the intestinal crypt were measured through the use of 10% neutral buffered formalin, light microscope and pigment of Haematoxylin and Eosin (H & E).

The abdominal cavity was opened in a transverse line incision. All inner organs of the slaughtered quails were detected and the intestine of individual quails was smoothly detached. At that time, small parts of the small intestine, around one centimeter length, were detached from the duodenum (under the pyloric-duodenal connection), jejunum (two centimeters center between the end-point of the duodenal loop and Meckel's diverticulum) and ileum (one cm front to ileocecal connection) (Choe et al., 2012). All small intestinal parts were fixed in ten percent neutral buffered formalin (pH 7, 20 – 24 °C) for three days (Caruso et al., 2012, Sabet Sarvestani et al., 2015), dry in increasing sequences of ethanol, cleared in xylene, set in paraffin blocks and then they were cut into 5μm slices with a microtome (WES WOX optic model 1090A, Italy). In individual quail, five slices that characterized the top observation of crypts and villus were carefully chosen to be analyzed. The slices were stained by (H&E) for typical histological evaluation.

Slides were studied by using light microscope (Optika, Italy); and measurements with photomicrographs were taken by using toupview 4.1 software (ToupTek Photonics, USA). The subsequent parameters of the small intestinal histomorphometric were estimated for all slices stained with the H&E technique: the length and width of the villus, the surface area of villi, as well as the intestinal crypt depth. The villi lengths were distinct from the tip to bottom and the widths were determined at the point in the middle of the length. Approaches through villus length and width at half tallness provided the villus surface area. The small intestinal crypts wwere measured as the space from the highest villus crypt to the muscularis mucosa (Figure 1). Dimensions were finished in μm, at X100 magnification.

Statistical analysis

Statistical analysis was done using Completely Randomized Design (CRD) SAS (2004), difference between treatments was done by Duncan's multiple rang test (1955) at (p≤ 0.05) and according to the equation model : -

$Y_{ij} = \mu + t_i + e_{ij}$ Since:

Y_{ij} = Value of Observations

μ = Average of Observations

t_i = Transaction Effect

e_{ij} = Impact of Experimental Error

RESULTS

Table (1) illustrates that there were no statistical differences in initial body weight between the four groups. However, significant (P<0.05) differences were observed in 1st, 2nd, 3rd, and 4th week with better body weight in organic acid treated groups as compared to the control group. In the fifth week, there have been no differences of statistical significance between the 4 groups in terms of the body weight.

As shown in Table 2, weight gain, feed consumption, and feed conversion ratio was unaffected (P>0.05) by the dietary organic acids in quails. However, weight gain in week 2 was significantly (P<0.05) improved in group 4 as compared to other groups.

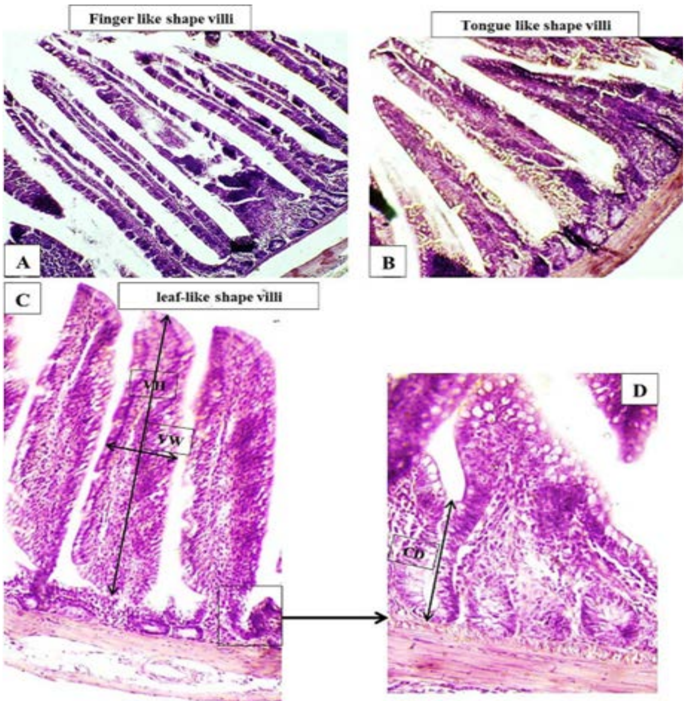

Figure 1: The illustrative histomorphometry of duodenum (A), jejunum (B) and ileum (C, D) at 35 days of age in quail, (x100) (VH: villus height, VW: villus width, CD: crypt depth (x400).

The mucosa of the small intestine was projected as elongated growth of villi was observed with finger-like shape that was formed in duodenum, tongue-shaped formed in the jejunum and leaf-like formed in the ileum (Figure 1). Table (3) showed that highest (P<0.05) villus length was recorded in group 2 of the duodenum; and group 3 of duodenum, jejunum and ileum in comparison to control

Table 1: The impact of adding both acetic and malic acids on the birds' body weight (g; mean ± standard error)

Groups	Initial weight	First week	Second week	Third week	Fourth week	Fifth week
Group 1	6.33± 0.33	18.33± 0.88^{AB}	61.66± 0.66^{B}	108.66± 0.66^{B}	151.33± 1.20^{B}	186±4.16
Group 2	7.00±0.57	18.33±0.33^{AB}	62.33±1.20^{B}	108.33±2.84^{B}	151.66±2.90^{B}	184.66±2.60
Group 3	7.66±0.33	16.66±1.76^{B}	61.66±0.66^{B}	109.00±0.57^{B}	153.00±2.00^{B}	194.33±8.25
Group 4	7.00±0.57	20.33±0.66^{A}	68.33±1.45^{A}	117.66±1.20^{A}	160.66±0.33^{A}	195.33±2.60

The different letters within the same column differ from each other at a probability level of p ≤0.05.
Group 1: the control group, which is free of any extra materials added.
Group 2: the group to which malic acid was added in drinking water by 0.25 gm/liter of distilled water (0.025 %).
Group 3: the group to which an acetic acid was added in drinking water by 0.25 ml /liter of distilled water (0.025 %).
Group 4: the group to which malic acid and acetic acid were added in drinking water by 0.025 % for each of them.

Table 2: The effect of adding malic and acetic acid on the weight gain (g), feed consumption (g) and feed conversion ratio in quails (mean ± standard error)

Variable	Week	G1	G2	G3	G4
Weight gain (g)	Week 1	12.00±1.00	11.33 ±0.33	10.66±1.76	14.33±0.66
	Week 2	43.33±1.20^{B}	44.00±1.15^{B}	45.00±0.66^{AB}	48.00±1.00^{A}
	Week 3	47.00±1.00	46.00±2.30	47.33±0.33	49.33±0.33
	Week 4	42.66± 0.88	43.33±0.88	44.00±2.08	41.33±1.33
	Week 5	34.66±3.17	33.00±0.57	41.33±9.83	34.66±2.90
Feed consumption (g)	Week 1	39.33±2.18	47.33±8.25	46.67±8.41	41.00±7.54
	Week 2	84.00± 6.02	88.00±8.71	88.33±5.92	95.66±6.74
	Week 3	125.67±6.64	129.67±11.02	130.67±7.42	136.00±3.60
	Week 4	114.00±8.18	129.33±14.88	122.67±5.17	134.33±4.05
	Week 5	126.00±8.14	123.00±2.08	126.66±4.37	121.66±4.97
Feed conversion ratio	Week 1	3.09±0.08	4.18±0.77	4.68±1.13	2.85±0.46
	Week 2	1.94± 0.17	1.98±0.14	1.96±0.12	1.99±0.17
	Week 3	2.67±0.14	2.82±0.26	2.75±0.15	2.75±0.08
	Week 4	2.66± 0.17	2.98±0.34	2.79±0.19	3.12±0.14
	Week 5	3.66±0.23	3.72±0.01	3.40±0.72	3.55±0.27

The different letters within the same row differ from each other at a probability level of p ≤0.05.
Group 1: the control group, which is free of any extra materials added.
Group 2: the group to which malic acid was added in drinking water by 0.25 gm/liter of distilled water (0.025 %).
Group 3: the group to which an acetic acid was added in drinking water by 0.25 ml /liter of distilled water (0.025 %).
Group 4: the group to which malic acid and acetic acid were added in drinking water by 0.025 % for each of them.

Table 3: The effect of adding malic and acetic acid on histological parameters of the small intestine of quails (mean ±standard error)

No. of Variable	Variable	Intestinal part	Group 1	Group 2	Group 3	Group 4
1	Villi length (µm)	**Duodenum**	935.88±43.90^{B}	1181.15±31.16^{A}	1244.19±27.97^{A}	819.19±5.26^{C}
		Jejunum	756.42±15.95^{B}	739.73±18.43^{B}	860.30±14.21^{A}	634.15±29.08^{C}
		Ileum	554.26±14.04^{B}	385.35±2.67^{C}	633.18±14.97^{A}	584.53±21.78^{B}
2	Villi width (µm)	**Duodenum**	103.65±3.48^{B}	126.65±5.27^{A}	123.64±6.48^{A}	120.03±5.26^{A}
		Jejunum	100.69±5.12^{A}	82.42±5.82^{A}	81.41±9.72^{A}	58.42 ±5.32^{B}
		Ileum	99.65±3.68^{A}	74.86±4.64^{B}	63.88±4.72^{C}	79.01±5.72^{B}
3	Crypt depth (µm)	**Duodenum**	30.62±0.93^{BC}	32.92±1.17^{B}	28.97±0.74^{C}	37.20±1.98^{A}
		Jejunum	24.11±1.21^{B}	22.59±0.71^{B}	22.20±1.50^{B}	34.47 ±1.78^{A}
		Ileum	18.73±0.24^{C}	23.66±1.44^{B}	27.39±1.10^{A}	22.70±1.83^{B}

4	Surface area (μm)	**Duodenum**	96994.36±5837.417^{B}	150247.99±8627.77^{A}	154280.60±9606.44^{A}	98197.86±4352.13^{B}
		Jejunum	76189.15±4124.92^{A}	61440.83±4303.87^{A}	70165.87 ±8762.48^{A}	37246.47±4285.75^{B}
		Ileum	55319.09±2598.16^{A}	28843.66±1804.39^{C}	40267.48±2853.61^{B}	45958.74±3602.32^{B}

1.F- value(df1-df2) = 40.475 (3-36) for variable 1
2.F- value(df1-df2) = 3.832 (3-36) for variable 2
3.F- value(df1-df2) = 7.57 (3-36) for variable 3
4.F- value (df1-df2) = 18.188 (3-36) for variable 4
The letters in each row denote differences that are statistically significant (P<0.05).

group. Similarly, statistically significant (P<0.05) decrease was observed in the villi length of duodenum and jejunum in group 4, and in group 2 in the ileum in comparison to the control group. From the other hand, Table (3) shows significant increase (P<0.05) in villi width of the duodenum in all organic acid treated groups as compared to the control, while in jejunum and ilum the change (P<0.05) was inconsistent.

Crypt depth was improved (P<0.05) in the duodenum and jejunum in the group 4, and for all the groups of ileum as compared to the control group. That was obvious in group 3 in comparison to the control group (Table 3). However, Table (3) further demonstrates increase (P<0.05) in both of the groups 2 and 3 in the villi surface area of the duodenum in comparison to the control group. Moreover, there have been no statistically significant differences (P>0.05) in group 4 in terms of villi surface area of the duodenum, and group 2 and 3 of jejunum in comparison to the control group. Likewise, there were significant decrease (P<0.05) in group 4 in villi surface area of the jejunum and also in all the groups of ileum and that was obvious in group 2 as compared with control group.

DISCUSSION

Our results demonstrates that there have been no differences of statistical significance in the weight of the body between the four groups in terms of the initial body weight. However, it was noticed that, with age, the fourth group had a significant increase in body weight compared to the other groups and this could be due to the role played by the acids concerning reducing the pH value in the digestive tract of birds, thus enhancing the numbers of beneficial bacteria in the intestinal flora. Consequently, the vital activity increases by improving the nutritional value of the nutrients included in the components of the ration through the secretion of digestive enzymes to proteins, fats and carbohydrates, as well as an increase in the amylase enzyme by lactobacillus bacteria, which is important in the process of starch analysis (Stanley et al., 1993). These results are compatible with the findings of (Moharrey and Mahzonieh, 2005). Also, the reason may be attributed to the increased absorption of nutritional compounds due to the increased villi width in the duodenum, as observed in our study (Table 3). In addition to that, there was a statistically significant increase in the rate of gaining weight for the fourth group, particularly in the age of two weeks. Moreover, no statistically significant differences were found between the 4 groups in the third, fourth and fifth weeks of age as a result of the halt of the increase in weight. These results were in agreement with that of (Frankenbach et al., 2001) and (Kaya and Tuncer, 2009).

Concerning the impact of organic acids upon the amount of feed consumption and the feed conversion factor, it seems that there is a slight effect on these two characteristics, as the effect was not statistically significant.

The morphology of the intestine is reflected through the chief pointer of standard gut histology (Laudadio et al., 2012). Findings of the current study illustrated that the mucosa of the small intestine was projected to an elongated growth evidenced by finger-shaped villi that was formed in the duodenum, tongue-like formed in the jejunum and leaf-like formed in the ileum. Also, the small intestine had a similar structure of the one observed in chickens (Ahmad et al., 2012). The present study demonstrated significant growths (P<0.05) in villi diameters (length and width) of all parts of the small intestine when maleic and acetic acids added at a concentration 0.025% in both the second and the third group respectively and that was more prominant in group three, compared to control group. This result is in agreement with the results of Ur Rehman et al. (2016) who discovered that adding the acetic acid to the feed of broiler, resulted in the development of epithelial cells and also improved the length and the area of the small intestine. The length and width diameters of the intestinal villi are morphometrical keys for growth and absorption capability (Markovic et al., 2009). Thus, this type of morphological alteration made in the intestine can have the consequence of growth and in intestinal absorption level and later the alteration in the quail performance (Oliveira et al., 2008). Thus synthetic acidifiers particularly acetic and maleic acid decrease the growth of numerous intestinal pathogenic bacteria. So, it declines the inflammation of the mucosa of the intestine, which increases the villus length and secretory function that ultimately improve digestion and absorption of the nutrients from mucosa (Iji and Tivey, 1998; Loddi et al., 2004; Pelicano et al., 2005). For example, the growth in villi length can lead to a growth in the whole luminal villus absorption area, which is considered important for digestive enzymes and greater transportation of nutrients at the villus surface (Tufarelli et al., 2010), but this is in contrast with the results of present study, as there were significant decreases (P<0.05) in group 4 in villi length of duodenum and jejunum; villi width of the jejunum and in all groups in villi width of ileum and that was evident when acetic acid alone added at a concentration of 0.025% and when maleic and acetic acid were added together at a concentration of 0.025% and 0.025% respectively, in comparison to the control group. This is in consistent with what was seen with the chickens that the size and density of villi along the mucosa of the intestine decreased caudally the same as the muscular mucosa and the size of the lumen (Gheisari et al., 2007). Also, the current research concluded that there were significant increase (P<0.05) in group 4 in the crypt depth of duodenum and jejunum and all groups in the crypts depth of ileum and that was obvious in group 3 in comparison to the control group. The efficient score of the small intestine was distinct by villus length and crypt depth (Laudadio et al., 2012). So, the results were in conformity with the ones of (Allahdo et al., 2018), who observed that chickens, which drank water that includes 2% of vinegar had longer villi, deeper crypt, and thinner muscular thickness compared to those which drank water without additions. The villi and crypts of the absorptive epithelium perform an essential role in the final phase of nutrient digestion and integration (Wang and Peng, 2008). The depth of the crypt and length of villi could be considered as helpful indicators for the intestinal mucosa functionality (Sharma and Schumacher, 2001). This study also showed improved villi surface area by supplementation of organic acids. Growing the villi length proposes an improved surface area that accomplished better absorption of existing nutrients (Caspary, 1992). Therefore, the use of synthetic acid decreased intestinal function and microbial load, thus, reducing inflammatory process at the intestinal mucosa and this enhanced villi length and the ability of secretion, digestion, and absorption of nutrients (Khan and Iqbal 2016). Absorption of nutrients and medications is augmented by the huge surface area that exists in the small intestine (Kararli, 1995). Changes in intestinal morphology can disturb nutrient metabolism capability and production (Laudadio et al., 2012). The growth of small intestine could be judged through the dimensions of the length of villus, width of villus, crypt depth and the area of the surface that exists for the purpose of digestion and absorption (Swatson et. al., 2002; Franco et. al., 2006).

CONCLUSION

In conclusion, this study demonstrated that addition of a mixture of acetic and malic acid can be beneficial for digestion and absorption of nutrients in all parts of the small intestine. This is achieved through the improved villi length, width, depth of the crypt and ultimately the villi surface area, resulting in increased body weight of quails.

ACKNOWLEDGMENTS

The authors express their gratitude for the efforts of the Department of Veterinary Public health and Department of Anatomy in the College of Veterinary Medicine, University of Mosul, to provide them with all facilities.

CONFLICT OF INTEREST

The author(s) declare that they have no competing interests regarding the publication of this paper.

NOVELTY STATEMENT

This is the first study conducted to find out the health benefits of acidification in the drinking water of quail birds through acids and the extent of its effect on productive characteristics, in addition to the remarkable improvement in the utilization of food through the increase in the length and width of the villi in the small intestine through the process of absorption.

AUTHORS CONTRIBUTION

All the author(s) contributed equally towards completion of this paper.

REFERENCES

Abbas G, Arshad M, Saeed M, Imran S, Kamboh AA, Al-Taey DK, Aslam MA, Imran MS, Ashraf M, Asif M, Tanveer AJ, Qureshi RAM, Arshad M, Niazi HAK, Tariq M, Abbas S (2022). An update on the promising role of organic acids in broiler and layer production. J. Anim. Health Prod. 10(3): 273-286. https://doi.org/10.17582/journal.jahp/2022/10.3.273.286

Attia, A.Y, Abdul El- Hamid, Hany F.Ellakany. Fulvia Bovera, Mohammed A.Al-Harth, Sharehan A. Ghazaly (2013). (Growing and Laying performance of Japanese Quail fed Diet Supplemented with Different concentrations of Acetic Acid. Italian J. Anim. Sci. 12: e37. https://doi.org/10.4081/ijas.2013.e37

Bauermann,U. (2006). (Essential oils in animal nutrition), feed magazine, 4, 19-21, 2006 .

Caruso M, Demonte A, Neves VA (2012). Histomorphometric study of role of lactoferrin in atrophy of the intestinal mucosa of rats. Health. 2012; 04(12): 1362–1370. https://doi.org/ 10.4236/health.2012.412198.

Caspary WF. (1992). Physiology and pathophysiology of intestinal absorption. Am. J. Clin. Nutr. 55:299S–308S.

https://doi.org/10.1093/ajcn/55.1.299s

Choe D. W., Loh Dr T. C., Foo H. L., Hair-Bejo M., Awis Q. S. (2012). Egg production, faecal pH and microbial population, small intestine morphology, and plasma and yolk cholesterol in laying hens given liquid metabolites produced by Lactobacillus plantarum strains. Brit. Poult. Sci. 53(1), 106-115. https://doi.org/10.1080/00071668.2012.659653

Davidson P.M (2001). Chemical preservative and natural antimicrobial compounds. Food microbiology .P.593 – 627 In : Ibid . USA .

Duncan D.B. (1955). Multiple range and multiple F. test. Biometrics, (11): 1-42. https://doi.org/10.2307/3001478

Franco J, Murakami AE, Natali M, et al. (2006). Influence of delayed placement and dietary lysine levels on small intestine morphometrics and performance of broilers. Braz. J. Poult. Sci. 8(4): 233–241. https://doi.org/10.1590/s1516-635x2006000400006.

Frankebach S.D., J. L snow, C.M. Parson, D.H,Baker (2001). The Effects of citric acid on the calcium and phosphorus requirements of chicks feed corn – soybean diets. Poult. Sci., 80: 783-788. https://doi.org/10.1093/ps/80.6.783

Gauthier R. (2002). Intestinal health , the key to productivity the case of organic acid XXVII convencion ANECA – WPDSA Puerto Vallarta, Jal. Mexico.

Gheisari A.A., M. Heidari, R.K. Kermanshahi, M. Togani S. (2007). Saraeian (Effect of dietary supplementation of protected organic acids on ileal microflora and protein digestibility in broiler chickens). In: proceedings of the 16 the European Symposium on poultry Nutrition Strasbourg, France .pp.519-522.

Iji, P. A, D. R. Tivey. (1998). Natural and synthetic oligosaccharide in broiler chicken diet. Worlds Poutl. Sci. J., 54: 129-143. https://doi.org/10.1079/WPS19980010

John Masuood Ahmad, C. S. Mamde, V. S. Patil, N. M. Dehaker (2012). Histomorphological Studies on the Small Intestine of Japanese Quail (*Coturnix coturnix japonica*). Indian J. Vet. Anat. 2012; 24 (2): 103-105.

Jovank L, S. Markov, D.Olivera, S.Sredanovic (2008). (Slavical Herbs and organic acids as an alternative for antibiotic growth – promoters). Archiva Zootech. 11:2 , 5- 11 .

Kararli TT (1995). Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm Drug Dispos. 16(5): 351–380, indexed in Pubmed: 8527686. https://doi.org/10.1002/bdd.2510160502

Kaya C.A., S. D. Tuncer (2009).The effect of an organic acid and etheric oil mixture on fattening performance , carcass quality and some blood parameters of broiler. J. Anim. Vet. Adv. 8,1:94-98.

Khan SH, Iqbal J. (2016). Recent advances in the role of organic acids in poultry nutrition. J. Appl. Anim. Res. 44:359–369. https://doi.org/10.1080/09712119.2015.1079527

Laudadio V, Passantino L, Perillo A, et al. Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. Poult Sci. 2012; 91(1): 265–270, https://doi.org/10.3382/ps.2011-01675, indexed in Pubmed: 22184453.

Lim CI, Kim HK, Heo KN, You AS, Choo HJ (2023). Effects of dietary supplementation of fermented garlic powder on productive performance, hatchability, and biochemical blood parameters of layer breeders. J. Anim. Health Prod. 11(1): 50-55. https://doi.org/10.17582/journal.jahp/2023/11.1.50.55

Loddi, M. M., V. M. B.Maraes, , I. S. O.Nakaghi, , F.Tucci, , M. I. Hannas and J. A Ariki. 2004. Mannan oligosaccharide and organic acids on performance and intestinal morphometric characteristics of broiler chickens. Proceedings of the 20th annual symposium. Supplement. 1, p. 45.

Markovi'c R, 'Sefer D, Krsti'c M, Petrujki'c B. (2009). Effect of different growth promoters on broiler performance and gut morphology. Arch. Med. Vet. 41:163–169. https://doi.org/10.4067/S0301-732X2009000200010

Moharrey A, M. Mahzonich (2005). Effect of malic on visceral characteristics and coliform counts in small intestine in the broiler and layer chickens. Int. J. Poult. Sci. 4 (10): 761 – 764. https://doi.org/10.3923/ijps.2005.761.764

Mulla ZS, Abdelhafeez MM (2022). Bacteriological status and prevalence of shiga toxin producing E. oli in bovine meat products with a reduction trial using acetic and citric acids. J. Anim. Health Prod. 10(3): 360-367. https://doi.org/10.17582/journal.jahp/2022/10.3.360.367

Mustafa, Mahbuba A.GH, Muhammed . Sallaiman, Lajan S (2014). Effect of acetic acid added to drinking water of two broiler strains on performance and small intestine histological . Diyala Agricult. Sciences J. , 6 (1) 1-82.

Oliveira MC, Rodrigues EA, Marques RH, Gravena RA, Guandolini GC, Moraes VMB. (2008). Performance and morphology of intestinal mucosa of broilers fed mannanoligosaccharides and enzymes. Arq. Bras Med. Vet. Zootec. 60:442–448. https://doi.org/10.1590/S0102-09352008000200025

Parviz Allahdo, Javad Ghodraty, Heydar Zarghi, Zohre Saadatfar, Hassan Kermanshahi, Mohammad Reza Edalatian Dovom. Effect of probiotic and vinegar on growth performance, meat yields, immune responses, and small intestine morphology of broiler chickens. Italian J. Anim. Sci. 17 (3): 675–685. https://doi.org/10.1080/1828051X.2018.1424570

Pelicano E., P.A. Souza, H.B.A. Soza; D.F. Figueiredo; M.M. Boiago; S.R. Carvahlo, V.F. Bordon, (2005). Intestinal mucosa development in broiler chickens feed growth promoters. Brazelian .J. Poult. Sci ., 7 (4) : 221- 229. https://doi.org/10.1590/S1516-635X2005000400005

Sabet Sarvestani F, Rahmanifar F, Tamadon A. (2015). Histomorphometric changes of small intestine in pregnant rat. Vet Res Forum. 2015; 6(1): 69–73. indexed in Pubmed: 25992254. https://doi.org/10.1155/2015/236436

Sas (2004). Sas user's guide. Statistics 2004, Edition 9.1 SAS .Institute Inc.cary , N.C.USA.

Sharma R., Schumacher U. (2001). Carbohydrate Expression in the Intestinal mucosa. Adv. Anat. Embryol. Cell Biol. 160(III-IX): 1-91. https://doi.org/10.1007/978-3-642-56704-9

Stanley V.G., Ojo, R. Woldensenbet S., Hutchinson D.H. (1993). (The use of saccharomyces cerevisiae to suppress the effect of aflatoxicosis in broiler chicks. Poult. Sci. 72: 1867-1872. https://doi.org/10.3382/ps.0721867

Swatson H, Gous R, Iji P, et al. Effect of dietary protein level, amino acid balance and feeding level on growth, gastrointestinal tract, and mucosal structure of the small intestine in broiler chickens. Anim Res. 2002; 51(6): 501–515. https://doi.org/10.1051/animres:2002038

Tufarelli V, Desantis S, Zizza S, et al.(2010). Performance, gut morphology and carcass characteristics of fattening rabbits as affected by particle size of pelleted diets. Arch Anim. Nutr. 64(5): 373–382, https://doi.org/10.1080/1745039X.2010.496945. indexed in Pubmed: 21114233.

Ur Rehman Z. U., A. U. Haq, N. Akram, M. E. A. El-Hack, M. Saeed, S. U. Rehman, C. Meng, M. Alagawany, Sayab, M. Dhama, K., C. Ding. (2016). Growth performance, intestinal histomorphology, blood hematology and serum metabolites of broilers chickens fed diet supplemented with graded levels of acetic acid. Pharmacol. 12(8): 874-883. https://doi.org/10.3923/ijp.2016.874.883

Wang JX, Peng KM. (2008). Developmental morphology of the small intestine of African ostrich chicks. Poult. Sci. 87(12): 2629–2635. https://doi.org/10.3382/ps.2008-00163.