EFFECT OF USING ORGANIC ACIDS AND ENZYMES ON PERFORMANCE OF JAPANESE QUAIL FED OPTIMAL AND SUB- OPTIMAL ENERGY AND PROTEIN LEVELS 1. MALIC ACID

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ABSTRACT:

An experiment was conducted to study the effectiveness of dietary malic acid supplementation as a growth promoter on Japanese quail performance, carcass characteristics, intestinal villi and pH, bacteria enumeration, blood parameters, digestibility coefficients and economical efficiency. A total number of 360 unsexed day-old Japanese quail chicks were equally divided into 4 groups of 6 replicates each. Two starter-grower corn-soybean meal (C-SBM) basal diets were formulated to contain 24 % CP & 2900 kcal ME /kg diet and 22 % CP & 2750 kcal ME /kg diet, respectively. Also, two layer C-SBM basal diets were formulated to contain 20 % CP & 2900 kcal ME /kg diet and 18 % CP & 2750 kcal ME /kg diet, respectively. Each of the 4 basal diets was either unsupplemented or supplemented with 0.05 % malic acid. Therefore, 4 experimental treatments were used in both starting-growing and laying periods. Each chick group fed one of the 4 experimental diets. At 35 days of age, a slaughter test was performed to determine carcass traits, edible giblets, lymphoid organs and intestinal villi, microflora count and pH. Blood samples were taken and assayed to determine some serum blood parameters. Digestibility trials were conducted to determine nutrients digestibility for starter-grower experimental diets. At laying period, egg number, weight, mass and production as well as feed intake and conversion were recorded. At the end of the 90-day period, egg samples were taken and broken out to determine internal egg quality and analysis. From nutritional and economical point of view, it was observed that using malic acid at a level of 0.05 % in Japanese quail diets containing sub-optimal energy and protein levels helped in reducing microflara count, particularly pathogens and in turn, improving quail performance and immunity. This can alleviate the financial pressure on the farmer.

Key words: Acidifier, organic acid, malic acid, malate, performance, intestinal pH, carcass, serum, egg quality, Japanese quail.

INTRODUCTION

Livestock performance and feed efficiency are closely interrelated with qualitative and quantitative microbial load of host animal, including load in alimentary tract and environment **(Garrido *et al.*, 2004)**. Antibiotic growth promoters (AGP) for poultry diets have been banned for use due to the possibilities of antibiotic residue, the development of drug-resistant bacteria and a reduction in the ability to cure these bacterial diseases in humans **(Jensen, 1998)**. Therefore, searching for alternative products that can be used in poultry feed and aid in growth promotion, feed utilization improvement, and maintenance of gut health are taking place.

Supplementing poultry diets with organic acids has become an important nutritional strategy aimed at improvement of performance and health status of

poultry fed diets devoid of AGP. Organic acids, as feed additives have received increasing attention as alternative AGP. It has made a tremendous contribution to the profitability in the intensive husbandry and providing people with healthy and nutritious poultry products **(Patten, and Waldroup, 1988)**. Organic acids may stimulate endogenous enzymes, regulate gut microbial flora and help in maintaining animal's health. The key basic principle on the mode of action of organic acids on bacteria is that nondissociated (non-ionised, more lipophilic) organic acids can penetrate the bacteria cell wall and disrupt the normal physiology of certain types of bacteria **(Dhawale, 2005)**.

Malic acid (MA), an alpha-hydroxy organic acid, is a colorless, crystalline compound, COOH·CH2·CHOH·COOH, that occurs naturally in a wide variety of unripe fruit, including apples. It is sometimes referred to as a fruit acid. It is also formed in metabolic cycles in plant and animal cells, including chickens. Peripheral malate derives from feed sources and from synthesis in citric acid or Krebs cycle located in cells' mitochondria **(Lehninger, 1978)**. Little literature was found on dietary MA effect in poultry and the evidence by which exogenous MA may affect on quail performance. Therefore, the purpose of the current study is to evaluate the effects of dietary MA supplementation on Japanese quail (*Coturnix Coturnix Japonica*) performance, carcass characteristics, intestinal villi and pH, bacteria enumeration as well as economical efficiency.

MATERIALS AND METHODS

Experimental birds and housing

Three hundred and sixty unsexed day-old Japanese quail chicks were used in a 35 day growing trial. Chicks were individually wing-banded, weighed, and randomly distributed into 4 experimental groups of similar mean body weight (7.62±0.05 g/bird) of 90 birds each, which consists of 6 replicates of 15 birds each. At 35 days of age, birds were transferred to layer quail cages for a 90 day laying trial.

Experimental diets

Two starter-grower corn-soybean meal (C-SBM) basal diets were formulated, from the same batches of components, to contain 24 % CP & 2900 kcal ME /kg diet (HPHE-diet) and 22 % CP & 2750 kcal ME /kg diet (LPLE-diet). Also, two layer C-SBM diets were formulated to contain 20 % CP & 2900 kcal ME /kg diet and 18 % CP & 2750 kcal ME /kg diet. Each of the 4 basal diets was either unsupplemented or supplemented with 0.05 % MA. Therefore, 4 experimental treatments were used in both starting-growing and laying periods. Each chick group fed one of the 4 experimental diets. The composition and chemical analysis of the experimental diets are shown in Table (1).

Management

Quail chicks were reared under similar management conditions. Ambient temperature was maintained at 34-36° C during the 1st week and weekly decreased by 5° C for the next 3 weeks. During the weeks 5 and 6 temperature was maintained at 20-22° C. Birds received continuous artificial daily lighting during growing trial and 17 h afterwards. Chicks were fed the starter-grower diets from 0 to 35 d and the layer diets from 35 to 125 d of age. Mash feed and clean fresh tap water were provided *ad liblitum*.

Table (1): Composition and calculated analysis of the experimental starter-grower and layer basal diets.

Ingredients	Percentage (%)			
	Starter-grower basal diets*		Layer basal diets*	
	B_1	B_2	B_1	B_2
Yellow Corn, ground	56.09	54.54	62.90	61.30
Soybean meal (44% CP)	33.00	32.50	19.22	21.02
Corn gluten meal (62% CP)	7.75	3.30	10.00	4.40
Wheat bran	0.00	6.80	0.00	5.52
Dicalcium phosphate	0.90	0.50	1.23	0.95
Limestone	1.30	1.50	5.68	5.80
Common salt (NaCl)	0.32	0.32	0.32	0.32
Premix**	0.30	0.30	0.30	0.30
DL-Methionine	0.08	0.10	0.07	0.13
L-Lysine	0.26	0.14	0.28	0.26
Total	100.00	100.00	100.00	100.00
Price (L.E./Ton) ***	1447	1390	1303	1287
Calculated analysis****				
CP %	24.09	22.05	20.00	18.05
ME (kcal/kg)	2903	2763	2907	2757
CF %	3.64	4.27	2.86	3.48
Ca %	0.80	0.80	2.50	2.50
Av. Phosphorus %	0.31	0.31	0.35	0.35
L-Lysine %	1.31	1.20	1.00	1.00
DL-Methionine %	0.50	0.46	0.45	0.45
Methionine + Cyst %	0.90	0.89	0.80	0.81

*Starter-grower and layer basal diets were assigned to 2 levels of MA (0 & 0.05%).

**Vitamins and minerals premix provides per kg of diet: 10000 IU Vit. A;. 1100 IU Vit. D_3, 1.1 mg Vit E; 0.02 mg Vit B_{12}; 1 mg Vit B_1; 0.16 mg Choline chloride; 3 mg Cu; 30 mg Fe; 40 mg Mn; 45 mg Zn and 3 mg Se.

***The Price (L.E./Ton) of B_1 and B_2 starter-grower basal diets were 1547 and 1490 as well as The Price of B_1 and B_2 layer basal diets were 1402 and 1337, respectively when MA was added.

****According to Feed Composition Tables for animal & poultry feedstuffs used in Egypt (2001).

Measurements and data collection

Growth performance:

Individual body weight (BW, g) and feed intake (FI, g/bird) were weekly recorded to determine body weight gain (BWG, g), feed conversion ratio (FCR, g feed/g gain), protein intake (PI), caloric intake (CI) protein conversion ratio, (PCR) and caloric conversion ratio (CCR). Mortality rate (MR) % was also calculated on weekly basis.

Carcass parameters

At the end of the starting-growing period (35 days), 48 birds (6 ♂+6 ♀/treatment) with BW similar to the mean were slaughtered to determine carcass characteristics. Obtained criteria were eviscerated carcass, dressing, breast and thigh weights. Abdominal fat was removed from gizzard and abdominal region and individually weighed for each carcass. Ovary-oviduct was carefully separated and accurately weighed. Edible giblets (liver, heart and gizzard) were individually separated and weighed. Lymphoid organs (thymus, bursa and spleen) were individually removed, weighed and calculated for each organ as % of live BW.

Villus height and width

Digesta from gastrointestinal tract were flushed at pH 7.4 to avoid damage to tissues. Intestinal samples of 1 cm in length were taken from the middle of each segment of the duodenum, jejunum, and ileum. Samples were then fixed in 10 % buffered neutral formaldehyde solution (pH 7.4), processed, and cut to 6-μm sections that were stained with hematoxylin and eosin and examined with a light microscope. A digital camera was used and villus height was measured from tip to villus bottom. Villus width was measured at villi bottom.

Bacteria enumeration and intestinal pH

At the time of slaughter test, 3 samples of ileum content for each treatment were taken. Total microflora, colibacillus and lactobacillus of ileum content were enumerated. Lactobacilli/colibacillus ratio was also calculated. The pH of intestinal contents was directly determined by pH-meter.

Blood serum parameters

At the time of slaughter test, 48 blood samples (6 ♂ & 6 ♀ / treatment) were withdrawn from wing vein and serum was separated by centrifugation for 10 minutes (3000 rpm) and stored in vials at –20 °C for later analysis. Frozen serum was thawed and assayed to determine, on individual bases, some biochemical parameters by using suitable commercial diagnostic kits and Atomic Absorption Spectrophotometer, following the same steps as described by manufactures. Calorimetric determination of serum total protein (TP, g/100 ml) was measured according to **Henry (1974)**. Albumin concentration (Alb, g/100 ml) was determined. Globulin concentration (Glo, g/100 ml) was calculated by the difference between TP and Alb, since the fibrinogen usually comprises a negligible fraction **(Sturkie, 1986)**. The Alb/Glo ratio was also calculated. Total lipids (TP, g/100 ml) and cholesterol (Cho, mg/100ml) were also determined.

Digestibility trials:

A total number of 24 adult ♂ quail of 6-wks old were selected at the end of the growing trial and individually housed in metabolic cages for carrying out 4 digestibility trials (6 ♂ /treatment) to determine the nutrient digestibility coefficient of dietary treatments. Digestibility trials lasted for 7 days, a 4-day preliminary period for adaptation to metabolic cages followed by a 3-day main collection period in which FI was offered on an *ad libitum* basis and excreta output was daily quantitatively collected for each ♂ over 3 consecutive days.

Egg traits and quality

Eggs were daily collected and weighed. Averages of egg number (EN), egg weight (EW), egg mass (EM) and FC per EM were weekly calculated per each replicate for 90-day laying period. Egg quality was assessed in 5 eggs collected per replicate during 3 days at the end of the 90-day period. Egg shape index (ESI) was determined according to **Stadleman (1977)**. Eggs were broken out and the liquid contents were put a side and shell plus membranes washed to remove adhering albumen. After drying, shell weight % was measured. Shell thickness (STh) was measured by using a micrometer as an average of 3 points (top, medial and base). Egg analysis including albumin protein % as well as yolk protein %, ether extract % and cholesterol (mg /gm yolk) were also performed according to **Washburn and Nix (1974)**.

Chemical and statistical analysis

Experimental diets and excreta were analyzed following procedures detailed by the Association of Official Analytical Chemists **(AOAC 1990)** for CP, CF, DM and EE. NFE was calculated by difference. Metabolizable energy (ME) of

experimental diets was calculated considering the ME values of different feed ingredients **(NRC, 1994)**. Fecal nitrogen was determined according to **Jakobsen *et al.* (1960)**.

Obtained data were expressed as means ± standard error and statistically analyzed by analysis of variance (ANOVA) as a factorial arrangement of 2 x 2 according to **Steel and Torrie (1980)**. Also, the General Linear Model (GLM) procedure of **SPSS** computer statistical program for MS Windows release 6.0 June 1993 was used. Significant means were ranked using Duncan's Range Test **(Duncan, 1955)**. Statistical significance level was tested at probability of $P \leq 0.05$.

RESULTS AND DISCUSSION

Growth performance:

The mean values of live performance parameters are shown in Table (2). Apart from MA, it was observed that feeding HPHE-diets resulted in significant increase in BW, BWG, FI, PI, CI, PCR and CCR values and significant decrease in MR % as compared to LPLE-diets. However, FCR was not significantly affected. Aside from diet type, feeding MA-supplemented diets gave significant improvement in BW, BWG, FCR, PCR and CCR as well as significant decrease in FI and MR % in comparison to MA-free diets. However, both PI and CI were not significantly affected. Supplementing MA to HPHE-diet had no significant effect on BW, BWG, FCR, PI, CI, PCR and CCR, whereas it significantly decreased FI and MR % as compared to the corresponding control diet. However, supplementing MA to LPLE-diet had significantly improved BW, BWG, FCR, PCR and CCR significantly decreased FI and MR %, but it had no significant effect on PI and CI.

These results are in agreement with those that have shown that organic acids have positive effects on poultry growth **(Chaveerach *et al.*, 2004)** and FI was decreased with increasing dietary propionic acid levels **(Cave, 1984).** The improvement in FC may be due to the acidic conditions that make the nutrients more available **(Boling *et al.*, 2001)** which monitors better performance. Oppositely, other results have shown that BW was not significantly affected by adding MA in drinking water **(Moharrery and Mahzonieh, 2005)** or dietary organic acid treatments **(Denli *et al.*, 2003).**

Carcass characteristics:

Data concerning carcass characteristics are presented in Table (3). Regardless of MA, it was noted that birds given HPHE-diet had significantly increased eviscerated carcass %, dressing % and breast %, but significantly decreased abdominal fat % and ovary-oviduct % as compared to those fed LPLE-diet. Irrespective of diet type, no significant influence was found due to MA supplementation on carcass parameters except for abdominal fat % that was significantly decreased and ovary-oviduct % that was significantly increased. Adding MA to HPHE-diet had no significant influence on eviscerated carcass %, dressing %, breast %, abdominal fat % and ovary-oviduct % as compared to the corresponding MA- free diet. However, supplementing MA to LPLE-diet had significantly increased eviscerated carcass %, dressing %, breast % and ovary-oviduct %, but it significantly decreased abdominal fat % as compared to the corresponding MA- free diet.

Edible giblets and lymphoid organs:

The results illustrated in Table (4) indicated that regardless of MA, feeding HPHE-diet had significantly increased liver % and heart %, however, gizzard

% was not significantly affected as compared to LPLE-diet. Regardless of diet type, MA supplementation caused no significant effect on edible giblets. Supplementing MA to HPHE- or LPLE-diets had no effect on edible giblets as compared to the corresponding MA-free diet. Concerning lymphoid organs data, it was noticed that either diet type or MA showed no significant effect, except for thymus gland that had significantly increased by MA supplementation. Adding MA to HPHE- or LPLE-diets had significantly increased lymphoid organs % as compared to the corresponding MA-free diet.

The present results are in agreement with those that have shown that liver % was not significantly affected by MA **(Moharrery and Mahzonieh, 2005)** and organic acids **(Denli *et al.* 2003)**. Thymus is a good indicator of immune function. **Shelat *et al.* (1997)** revealed that thymus size is a sensitive indicator of health and acute or chronic stress response.

Villus height and width:

The mean of intestinal villus height and width are summarized in Table (5). Apart from MA, diet type caused no significant effect on villus height and width in different intestinal segments. Irrespective of diet type, MA supplemented-diets had significantly increased intestinal villus height and width in different intestinal segments as compared to MA-free diets. Supplementing MA to HPHE- or LPLE-diets had significantly increased intestinal villus height and width in different intestinal segments as compared to the corresponding MA-free diets.

Bacteria enumeration and intestinal pH:

The mean of total microflora count, colibacillus, lactobacillus and lactobacillus/colibacillus ratio of the ileum content as well as intestinal pH are given in Table (6). Regardless of MA, diet type caused no significant effect on total microflora count, colibacillus and lactobacillus and lactobacillus/ colibacillus ratio of the ileum content as well as intestinal pH. Irrespective of diet type, MA supplementation resulted in significant decrease in total microflora count, colibacillus count and intestinal pH as well as significant increase in lactobacillus count, lactobacillus/colibacillus ratio as compared to MA-free diets. Supplementing MA to HPHE- or LPLE-diets resulted in significant decrease in total microflora count, colibacillus count and intestinal pH as well as significant increase in lactobacillus count, lactobacillus/ colibacillus ratio as compared to the corresponding MA-free diets.

These results are in agreement with those of **Moharrery and Mahzonieh, (2005)** who found that E. coli count was significantly decreased by MA. This due to organic acids can inhibit growth of many bacteria and toxin-producing molds **(Roy, 2002)**. Intestinal pH was not affected by formic and propionic acids **(Thompson and Hinton, 1997).** The acidic pH allows establishment of microorganisms, particularly Lactobacillus spp **(Sarra *et al.*, 1985)** and prevents E. coli growth and these conditions make the absorptive area more beneficial **(Dofing and Gottschal, 1997).**

Table (2): Effect of dietary treatments on performance of growing Japanese quail at 0 – 5 weeks of age.

Treatments		Initial BW (g/bird)	Final BW (g/bird)	BWG (g/bird/35 d)	FI (g/bird/35 d)	FCR (feed: gain)	PI (g/bird/d)	CI (kcal/bird/d)	PCR (protein: gain)	CCR (kcal: gain)	MR (%)
Starter-Grower Diet	MA (%)										
24% CP&2900 kcal	0	7.64±0.06	190.25±2.70^{a}	182.61±1.21^{a}	521.11±2.88^{a}	2.85±0.10^{b}	3.57±0.04^{a}	43.18±2.12^{a}	0.68±0.01^{a}	8.28±0.06^{a}	8.89^{b}
	0.05	7.58±0.05	192.37±3.00^{a}	184.79±1.32^{a}	500.20±5.01^{b}	2.71±0.07^{b}	3.43±0.02^{a}	41.45±2.34^{a}	0.65±0.04^{a}	7.85±0.09^{a}	2.22^{c}
22% CP&2750 kcal	0	7.61±0.03	155.10±2.90^{c}	147.49±1.24^{c}	468.51±3.27^{c}	3.18±0.12^{a}	2.94±0.01^{b}	36.81±2.29^{b}	0.70±0.02^{a}	8.74±0.08^{a}	11.11^{a}
	0.05	7.66±0.07	176.75±2.22^{b}	169.09±1.29^{b}	447.89±4.10^{d}	2.65±0.04^{b}	2.82±0.03^{b}	35.19±2.40^{b}	0.58±0.02^{b}	7.28±0.11^{b}	3.33^{c}
24% CP&2900 kcal		7.61±0.09	191.31±3.12^{A}	183.70±1.40^{A}	510.66±4.16^{A}	2.78±0.09	3.50±0.05^{A}	42.31±2.04^{A}	0.67±0.03^{A}	8.06±0.05^{A}	2.56^{B}
22% CP&2750 kcal		7.64±0.04	165.93±3.08^{B}	158.29±1.27^{B}	458.20±4.21^{B}	2.89±0.11	2.88±0.04^{B}	36.00±2.10^{B}	0.64±0.04^{B}	7.96±0.09^{B}	7.22^{A}
0		7.63±0.03	172.68±2.89^{B}	165.05±1.33^{B}	494.81±3.88^{A}	3.00±0.13^{A}	3.25±0.06	39.99±2.17	0.69±0.02^{A}	8.48±0.10^{A}	10.00^{A}
0.05		7.62±0.07	184.56±3.00^{A}	176.94±1.24^{A}	474.05±2.94^{B}	2.68±0.06^{B}	3.12±0.03	38.32±2.08	0.62±0.02^{B}	7.58±0.07^{B}	2.78^{B}

Means in the same column having different letters are significantly different at p≤0.05.

Table (3): Effect of dietary treatments on carcass characteristics of Japanese quail at 5 weeks of age.

Treatments		(% of BW)					
Starter-Grower Diet	MA (%)	BW (g/bird)	Eviscerated carcass	Dressing*	Breast	Abdominal fat	Ovary-oviduct
24% CP&2900 kcal	0	188.12±2.10^{a}	67.50±2.12^{a}	74.54±0.37^{a}	36.60±1.12^{a}	2.16±0.02^{b}	7.28±0.19^{a}
	0.05	191.22±2.04^{a}	67.66±2.30^{a}	73.74±0.91^{a}	36.90±1.04^{a}	2.01±0.04^{b}	7.41±0.22^{a}
22% CP&2750 kcal	0	152.00±2.35^{c}	58.19±1.96^{c}	64.00±0.46^{c}	25.11±1.10^{c}	3.10±0.01^{a}	4.10±0.11^{c}
	0.05	173.62±3.02^{b}	62.47±2.00^{b}	68.55±0.67^{b}	30.96±1.02^{b}	2.11±0.03^{b}	5.23±0.09^{b}
24% CP&2900 kcal		189.67±2.10^{A}	66.58±1.88^{A}	73.64±0.80^{A}	36.75±1.06^{A}	2.09±0.01^{B}	3.71±0.19^{B}
22% CP&2750 kcal		162.81±2.66^{B}	60.33±2.07^{B}	66.28±0.71^{B}	28.04±1.00^{B}	2.61±0.04^{A}	4.67±0.17^{A}
0		170.06±2.00^{B}	62.35±2.03	68.78±0.94	30.86±1.08	2.63±0.01^{A}	5.69±0.15^{B}
0.05		182.42±2.21^{A}	64.57±2.00	71.16±0.90	27.86±1.05	2.06±0.03^{B}	6.32±0.18^{A}

* Dressing % = [(Carcass weight + Giblets weight) / (Pre-slaughter weight)] x 100.

Means in the same column having different letters are significantly different at p≤0.05.

Table (4): Effect of dietary treatments on edible giblets and lymphoid organs % of Japanese quail at 5 weeks of age.

Treatments		Edible giblets (%)			Lymphoid organs (%)		
Starter-Grower Diet	MA (%)	Liver (%)	Heart (%)	Gizzard (%)	Thymus (%)	Bursa (%)	Spleen (%)
24% CP&2900 kcal	0	3.03±0.13^{a}	1.00±0.05^{a}	3.01±0.14	0.20±0.02^{b}	0.06±0.01^{b}	0.08±0.03^{b}
	0.05	3.01±0.17^{a}	1.04±0.07^{a}	3.03±0.11	0.36±0.01^{a}	0.17±0.03^{a}	0.13±0.01^{a}
22% CP&2750 kcal	0	2.00±0.15^{b}	0.79±0.04^{b}	3.02±0.16	0.18±0.03^{b}	0.05±0.02^{b}	0.06±0.02^{b}
	0.05	2.22±0.10^{b}	0.82±0.08^{b}	3.04±0.19	0.38±0.03^{a}	0.14±0.01^{a}	0.12±0.02^{a}
24% CP&2900 kcal		3.02±0.10^{A}	1.02±0.03^{A}	3.02±0.09	0.28±0.01	0.12±0.02	0.11±0.02
22% CP&2750 kcal		2.11±0.18^{B}	0.81±0.07^{B}	3.03±0.21	0.28±0.03	0.10±0.02	0.09±0.01
	0	2.52±0.16	0.89±0.05	3.02±0.06	0.19±0.02^{B}	0.06±0.01	0.07±0.01
	0.05	2.62±0.14	0.93±0.06	3.04±0.14	0.37±0.02^{A}	0.16±0.02	0.13±0.02

Means in the same column having different letters are significantly different at p≤0.05.

Table (5): Effect of dietary treatments on the intestinal villi of growing Japanese quail at 5 weeks of age

Treatments		Intestinal segment					
		Duodenum		Jejunum		Ileum	
Starter-Grower Diet	MA (%)	Villus height (µm)	Villus Width (µm)	Villus height (µm)	Villus Width (µm)	Villus height (µm)	Villus Width (µm)
24% CP&2900 kcal	0	447.2±2.10^{b}	100.3±1.11^{b}	323.8±3.01^{b}	91.2±2.12^{b}	205.7±2.13^{b}	90.3±1.15^{b}
	0.05	486.1±5.02^{a}	124.5±3.03^{a}	361.1±4.00^{a}	113.9±3.03^{a}	246.2±2.04^{a}	104.6±1.09^{a}
22% CP&2750 kcal	0	454.4±4.00^{b}	105.1±2.23^{b}	317.6±2.13^{b}	96.6±2.10^{b}	213.5±1.08^{b}	94.1±1.10^{b}
	0.05	479.6±3.22^{a}	118.7±4.02^{a}	355.3±5.04^{a}	116.7±1.22^{a}	252.1±1.04^{a}	110.4±1.31^{a}
24% CP&2900 kcal		466.65±4.54	112.40±2.00	342.45±4.11	102.56±2.13	225.95±2.10	97.45±1.19
22% CP&2750 kcal		467.00±3.87	111.90±2.23	336.15±3.46	106.65±2.06	232.80±2.02	102.25±1.14
	0	450.80±3.70^{B}	102.70±2.17^{B}	320.70±3.80^{B}	93.90±2.11^{B}	209.60±2.15^{B}	92.20±1.07^{B}
	0.05	482.85±5.00^{A}	121.60±3.14^{A}	358.20±3.32^{A}	115.30±2.09^{A}	249.15±2.04^{A}	107.50±1.11^{A}

Means in the same column having different letters are significantly different at p≤0.05.

Table (6): Effect of dietary treatments on total microflora count, colibacillus and lactobacillus and their ratio of the ileum content as well as intestinal pH of Japanese quail at 5 weeks of age.

Treatments		Microflora count (Log No/g)			Lactobacillus Colibacillus ratio	Intestinal pH
Starter-Grower Diet	MA (%)	Total Microflora count	Colibacillus	Lactobacillus		
24% CP&2900 kcal	0	10.83±0.12^{a}	6.21±0.10^{a}	4.40±0.18^{b}	0.71±0.12^{b}	6.70±0.09^{a}
	0.05	8.21±0.09^{b}	4.53±0.12^{b}	6.11±0.15^{a}	1.35±0.10^{a}	6.20±0.07^{b}
22% CP&2750 kcal	0	11.03±0.14^{a}	6.33±0.13^{a}	4.31±0.10^{b}	0.68±0.11^{b}	6.90±0.06^{a}
	0.05	8.32±0.13^{b}	4.37±0.12^{b}	6.24±0.12^{a}	1.43±0.15^{a}	6.10±0.03^{b}
24% CP&2900 kcal		9.52±0.07	5.37±0.06	5.26±0.14	1.03±0.13	6.45±0.08
22% CP&2750 kcal		9.68±0.14	5.35±0.11	5.28±0.11	1.06±0.14	6.50±0.05
	0	10.93±0.09^{A}	6.27±0.04^{A}	4.36±0.10^{B}	0.70±0.11^{B}	6.80±0.04^{A}
	0.05	8.27±0.15^{B}	4.45±0.09^{B}	6.18±0.10^{A}	1.39±0.08^{A}	6.15±0.04^{B}

Means in the same column having different letters are significantly different at p≤0.05.

Blood serum parameters:

Results concerning TP, Alb, Glo, Alb/Glo ratio, TL and Cho are shown in Table (7). There were no significant differences in either TP or Cho among different treatments. Irrespective of MA, HPHE-diet caused significant increase in Alb and Alb/Glo ratio and significant decrease in Glo and TL. Regardless of diet type, MA supplementation resulted in no significant differences among all studied traits. Supplementing MA to HPHE-diet had similar Alb, Glo, Alb/Glo ratio and TL values as compared to the corresponding MA-free diet. The same trend was observed in case of supplementing MA to LPLE-diet.

Table (7): Effect of dietary treatments on some serum blood parameters of growing Japanese quail at 5 weeks of age.

Treatments		TP (g/100 ml)	Alb (g/100 ml)	Glo (g/100 ml)	Alb/Glo ratio	TL (g/100 ml)	Cho (mg/100 ml)
Starter-Grower Diet	MA (%)						
24% CP& 2900 kcal	0	4.40±0.05	1.91±0.10^{a}	2.49±0.17^{b}	0.77±0.16^{a}	1.53±0.14^{b}	110.00±7.10
	0.05	4.42±0.11	1.86±0.13^{a}	2.56±0.13^{b}	0.73±0.14^{a}	1.56±0.12^{b}	107.11±6.21
22%CP& 2750 kcal	0	4.40±0.05	1.00±0.10^{b}	3.40±0.11^{a}	0.29±0.02^{b}	2.00±0.11^{a}	109.14±5.16
	0.05	4.43±0.10	1.07±0.14^{b}	3.36±0.19^{a}	0.45±0.16^{b}	1.96±0.19^{a}	106.24±6.96
24% CP&2900 kcal		4.41±0.09	1.89±0.06^{A}	2.52±0.13^{B}	0.75±0.04^{A}	1.55±0.14^{B}	108.56±5.43
22% CP&2750 kcal		4.42±0.10	1.04±0.08^{B}	3.38±0.11^{A}	0.31±0.09^{B}	1.98±0.13^{A}	107.69±8.00
	0	4.40±0.07	1.46±0.04	2.94±0.14	0.50±0.12	1.77±0.05	109.57±4.94
	0.05	4.43±0.12	1.47±0.11	2.96±0.05	0.50±0.10	1.76±0.07	106.68±6.11

Means in the same column having different letters are significantly different at p ≤ 0.05.

Nutrients digestibility coefficients:

Data regarding digestibility coefficients of CP, CF, EE and NFE values for experimental starter-grower diets are given in Table (8). There were no significant differences in CF and NFE digestibilities among different treatments. Apart from MA, HPHE-diet caused significant increase in CP and EE digestibility as compared to LPLE-diet. Away from diet type, MA supplementation caused significant increase for only CP digestibility. Supplementing MA to HPHE-diet had similar CP and EE digestibility as

compared to the corresponding MA-free diet. However, supplementing MA to LPLE-diet caused significantly increase in CP and similar EE digestibility as compared to the corresponding MA-free diet

In general, the improvement due to adding MA may be attributed to improving intestinal microbial balance. In other words, MA helps to keep the intestinal tract healthy and when the epithelial tissue is healthy, there is improved and better absorption of all nutrients **(Kaisths *et al.*, 1996).**

Economical efficiency:

Economical evaluation parameters for MA supplementation in Japanese quail diets varying in CP and ME in terms of feeding cost of the experimental diets, net revenue, economical efficiency (EE_f) and relative economical efficiency (REE_f) of meat production are listed in Table (9).

Taking MR % into account, results showed that MA-supplemented diets gave higher REE_f % than MA-free diets. Also, supplementing MA to HPHE-diet failed to increase REE_f % as compared to the corresponding MA-free diet. The highest REE_f % was obtained by supplementing MA to LPLE-diet. This may be due to FCR improvement and low FI for birds fed this experimental diet. Other explanation is the beneficial effect of MA which improved absorption of nutrients and depressed harmful bacteria that causes growth depression.

Table (8): Effect of dietary treatments on the digestibility coefficients.

Treatments		Digestibility coefficients (%)			
Starter-Grower Diet	MA (%)	CP	CF	EE	NFE
24% CP&2900 kcal	0	90.01±1.16^{a}	20.09±1.12	73.52±1.31^{a}	78.20±1.21
	0.05	90.05±1.14^{a}	20.11±1.14	73.66±1.23^{a}	78.14±1.10
22% CP&2750 kcal	0	79.60±1.10^{c}	20.13±1.33	68.04±1.11^{b}	78.17±1.34
	0.05	84.36±1.30^{b}	20.07±1.28	67.19±1.34^{b}	78.16±1.13
24% CP&2900 kcal		90.03±1.22^{A}	20.10±1.10	73.59±1.20^{A}	78.17±1.08
22% CP&2750 kcal		81.98±1.18^{B}	20.10±1.07	67.62±1.32^{B}	78.17±1.05
	0	84.81±1.37^{B}	20.11±1.23	70.78±1.13	78.19±1.21
	0.05	87.21±1.06^{A}	20.09±1.20	70.43±1.17	78.15±1.31

Means in the same column having different letters are significantly different at $p \leq 0.05$.

Laying performance:

Results concerning laying performance in terms of EP %, EN, EW, EM, FI and FCR values are shown in Table (10). Irrespective of MA, HPHE-diet caused significant improvement in EP %, EN, EW, EM and FCR as well as significant decrease in FI as compared to LPLE-diet. These results are in harmony with those of **Abdel-Rahman (1993)**; **Shrivastav *et al.*, (1993); Zanaty *et al.* (2001); Yakout *et al.* (2004)** and **Garcia *et al.*, (2005)** who reported that EP, EW, EM and FCR were improved with increasing dietary CP level. However, **Garcia *et al.*, (2005)** reported that FI was not significantly affected by dietary CP level.

Regardless of diet type, MA supplementation caused significant improvement in EP % and FCR, non significant differences in EN, EW and EM as well as significant decrease in FI. Supplementing MA to HPHE-diet had similar EP %, EN, EW, EM and FCR to the corresponding MA-free diet. The only exception was FI that was significantly decreased as compared to the corresponding MA-free diet. On the other hand, supplementing MA to LPLE-

diet caused significantly improvement in EP %, EW, EM and FCR except for FI that was significantly decreased as compared to the MA-free diet.

Egg quality:

Data regarding egg quality in terms of ESI, SG, STh, shell (S) %, yolk (Y) % and albumin (Alb) % are presented in Table (11). There were no significant differences in SG, Y % and Alb % among different treatments. These results are in a relative harmony with the results of **Garcia *et al.*, (2005)** who reported that dietary CP levels had no effect on Y %. On the contrary, increasing CP level increased Y % and reduced Alb % (Akbar ***et al.*, 1983),** increased Y % **(Yakout *et al.*, 2004)** and decreased Y % **(Zanaty, 2006)**.

Regardless of MA, HPHE-diet caused significant increase in ESI, STh and S %. Similar observations have been reported by **Yakout *et al.* (2004)** and **Zanaty (2006)** who found that STh was significantly increased with increasing CP. This may be due to the increase in EW or the enhancing of Ca deposition in the shell matrix.

Irrespective of diet type, MA supplementation caused significant increase in ESI, STh and S %. Supplementing MA to HPHE-diet had similar ESI, STh and S % to the corresponding MA-free diet. However, supplementing MA to LPLE-diet caused significant increase in ESI, STh and S % as compared to the corresponding MA-free diet.

Egg analysis:

Results concerning egg analysis in terms of albumin protein (Alb_p) %, yolk protein (Y_p) %, yolk ether extract (Y_{EE}) % and yolk cholesterol (Y_{Cho}) are shown in Table (12). Regardless of MA, HPHE-diet caused significant increase in Y_p % and significant decrease in Y_{EE} % and Y_{Cho} %. These results are in agreement with previous studies of **Andersson (1979); Akbar (1983)** and **Garcia *et al.*, (2005)** who reported that Y_p contents increased with higher dietary CP levels.

Irrespective of diet type, MA supplementation caused significant decrease in Y_{Cho} %. Supplementing MA to HPHE-diet caused similar Y_p % and Y_{EE} % as well as significant decrease in Y_{Cho} % as compared to the corresponding MA-free diet. The same trend was observed in case of supplementing MA to LPLE-diet.

From nutritional and economical point of view, it could be concluded that using MA at a level of 0.05 % in Japanese quail diets containing sub-optimal energy and protein levels helped in reducing microflara count, particularly pathogens and in turn, improving quail performance and immunity. This can alleviate the financial pressure on the farmer. If MA is used correctly along with nutritional, managerial and biosecurity measures, it can be a powerful tool in maintaining the health of the gastrointestinal tract, thus improving quail zootechnical performances.

Table (9): Input-output analysis and economical efficiency ratio of experimental treatments during the starter-growing period.

Treatments		Livability*	Total FI (kg/ treatment)	Total FI price (L.E / treatment)**	Total BW gain (kg/ treatment)	Total BW gain price (L.E / treatment)***	Net revenue (LE / treatment)	EE_f****	REE_f***** (%)
Starter-Grower Diet	MA (%)								
24% CP&2900 kcal	0	91.11	42.73	61.82	14.97	224.61	162.79	2.63	100.00
	0.05	97.78	44.02	68.09	16.26	243.92	175.83	2.58	98.06
22% CP&2750 kcal	0	88.89	37.48	52.10	11.80	176.99	124.89	2.40	91.03
	0.05	96.67	38.97	58.07	14.71	220.66	162.59	2.80	106.33
24% CP&2900 kcal		97.44	43.41	64.98	15.61	234.22	169.24	2.60	98.91
22% CP&2750 kcal		92.78	38.26	55.09	13.22	198.26	143.17	2.60	98.69
	0	90.00	40.08	56.85	13.37	200.54	143.69	2.53	95.98
	0.05	97.22	41.48	62.98	15.48	232.23	169.25	2.69	102.05

* Livability = 100 – MR %

**According to the local market price of feed ingredients at the experimental time.

***According to the local market price of one kg live body weight which was 15 L.E at the experimental time.

****EE_f: Economical efficiency, net revenue per unit of total feed cost.

*****Relative economic efficiency, assuming that the control treatment = 100 %.

Table (10): Effect of dietary treatments on performance of laying Japanese quail from 8 to 20 weeks of age.

Treatments		EP (%)	EN (No./hen/day)	EW (g)	EM (g/hen/day)	FI (g/hen/day)	FCR (g feed/g egg)
Layer Diet	MA (%)						
20% CP&2900 kcal	0	82.07 ± 3.22^{a}	0.82 ± 0.02^{a}	10.10 ± 0.06^{a}	8.28 ± 0.04^{a}	20.24 ± 0.21^{b}	2.44 ± 0.02^{bc}
	0.05	81.78 ± 2.44^{a}	0.82 ± 0.03^{a}	10.12 ± 0.09^{a}	8.30 ± 0.02^{a}	18.01 ± 0.37^{c}	2.17 ± 0.01^{c}
18% CP&2750 kcal	0	72.05 ± 4.04^{c}	0.75 ± 0.04^{b}	7.93 ± 0.04^{c}	5.95 ± 0.02^{c}	22.50 ± 0.22^{a}	3.78 ± 0.02^{a}
	0.05	78.09 ± 3.14^{b}	0.78 ± 0.03^{b}	9.00 ± 0.03^{b}	7.02 ± 0.02^{b}	20.04 ± 0.32^{b}	2.85 ± 0.03^{b}
20% CP&2900 kcal		81.93 ± 2.91^{A}	0.82 ± 0.05^{A}	10.11 ± 0.05^{A}	8.29 ± 0.05^{A}	19.13 ± 0.20^{B}	2.31 ± 0.01^{B}
18% CP&2750 kcal		75.07 ± 2.20^{B}	0.77 ± 0.07^{B}	8.47 ± 0.07^{B}	6.52 ± 0.07^{B}	21.27 ± 0.27^{A}	3.26 ± 0.03^{A}
	0	77.06 ± 3.09^{B}	0.79 ± 0.04	9.02 ± 0.04	7.13 ± 0.04	21.37 ± 0.30^{A}	3.00 ± 0.02^{A}
	0.05	79.94 ± 2.70^{A}	0.80 ± 0.06	9.56 ± 0.06	7.65 ± 0.09	19.03 ± 0.12^{B}	2.49 ± 0.01^{B}

Means in the same column having different letters are significantly different at $p\leq0.05$.

Table (11): Effect of dietary treatments on egg quality of laying Japanese quail from 8 to 20 weeks of age.

Treatments		ESI	SG	STh (μ)	% of EW		
Layer Diet	MA (%)				S	Y	Alb
20% CP&2900 kcal	0	78.00±0.21^{a}	1.03±0.001	321.30±0.02^{a}	10.44±0.11^{a}	30.09±0.23	59.47±0.81
	0.05	79.00±0.18^{a}	1.08±0.003	321.81±0.01^{a}	10.51±0.10^{a}	30.32±0.41	60.40±0.92
18% CP&2750 kcal	0	65.00±0.21^{c}	1.02±0.002	200.10±0.03^{b}	9.00±0.14^{c}	33.54±0.32	56.78±0.74
	0.05	73.00±0.20^{b}	1.10±0.002	314.63±0.01^{a}	9.60±0.12^{b}	33.21±0.43	57.09±0.61
20% CP&2900 kcal		78.50±0.16^{A}	1.06±0.03	321.56±0.04^{A}	10.48±0.07^{A}	30.21±0.23	59.94±0.55
18% CP&2750 kcal		69.00±0.19^{B}	1.06±0.02	257.37±0.03^{B}	9.30±0.13^{B}	33.38±0.20	56.94±0.74
0		71.50±0.24^{B}	1.03±0.01	260.70±0.01^{A}	9.72±0.11^{B}	31.82±0.24	58.13±0.69
0.05		76.00±0.11^{A}	1.09±0.03	318.22±0.01^{B}	10.06±0.09^{A}	31.77±0.20	58.75±0.71

Means in the same column having different letters are significantly different at $p \leq 0.05$.

Table (12): Effect of dietary treatments on egg analysis of laying Japanese quail from 8 to 20 weeks of age.

Treatments		Alb_p (%)	Y_p (%)	Y_{EE} (%)	Y_{Cho} (mg/gm yolk)
Layer Diet	MA (%)				
20% CP&2900 kcal	0	79.68±0.80	31.62±0.53^{a}	57.89±0.71^{b}	24.03±0.47^{a}
	0.05	79.71±0.78	31.71±0.77^{a}	57.99±0.66^{b}	20.71±0.78^{b}
18% CP&2750 kcal	0	79.52±0.67	29.84±0.91^{b}	60.41±0.58^{a}	26.05±0.83^{a}
	0.05	79.68±0.51	29.99±0.66^{b}	60.62±0.82^{a}	23.06±0.50^{b}
20% CP&2900 kcal		79.70±0.49	31.67±0.72^{A}	57.94±0.52^{B}	22.36±0.39^{B}
18% CP&2750 kcal		79.60±0.91	29.92±0.60^{B}	60.52±0.90^{A}	24.56±0.61^{A}
	0	79.60±0.55	30.73±0.81	59.15±0.76	25.04±0.48^{A}
	0.05	79.70±0.70	30.85±0.73	59.31±0.80	21.89±0.33^{B}

Means in the same column having different letters are significantly different at $p \leq 0.05$.

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تأثير استخدام الأحماض العضوية والأنزيمات على أداء السمان الياباني المغذى على مستويات مثلى وتحت المثلى من الطاقة والبروتين ١- حمض الماليك

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أستخدم فى هذه الدراسة ٣٦٠ كتكوت سمان ياباني غير مجنس عمر يوم تم توزيعها بالتساوى على ٤ معاملات غذائية (٦ مكررات/معاملة) استمرت حتى عمر ٢٠ أسبوع وذلك بهدف معرفة تأثير إضافة حمض الماليك على أداء النمو والأداء الإنتاجى وبعض صفات الذبيحة ومقاييس الدم وكذا الكفاءة الاقتصادية للسمان الياباني.

تم تكوين عليقتين نمو (كنترول) **الأولى** تحتوى على ٢٤ ٪ بروتين خام و٢٩٠٠ كيلو كالورى طاقة ممثلة/كجم علف لتغطى الاحتياجات الغذائية للسمان الياباني طبقا للمجلس القومى الأمريكى للبحوث (NRC) سنة ١٩٩٤ **والثانية** تحتوى على ٢٢ ٪ بروتين خام و٢٧٥٠ كيلو كالورى طاقة ممثلة / كجم علف وذلك لتغذية الكتاكيت خلال فترة النمو (١-٣٥ يوم). تم تكوين عليقتين بياض (كنترول) **الأولى** تحتوى على ٢٠ ٪ بروتين خام و٢٩٠٠ كيلو كالورى طاقة ممثلة / كجم علف لتغطى الاحتياجات الغذائية للسمان الياباني طبقا للمجلس القومى الأمريكى للبحوث (NRC) سنة ١٩٩٤ **والثانية** تحتوى على ١٨٪ بروتين خام و٢٧٥٠ كيلو كالورى طاقة ممثلة/كجم علف وذلك لتغذية الكتاكيت خلال فترة البياض (٦-٢٠ أسبوع). تم إضافة أو عدم إضافة ٠,٠٥٪ حمض الماليك إلى علائق النمو والبياض الكنترول وبذلك يكون هناك ٤ معاملات غذائية فى كل من فترة النمو والبياض.

فى نهاية فترة النمو (عمر ٣٥ يوم) تم ذبح ١٢ طائر (٦ إناث + ٦ ذكور) من كل معاملة (أنثى + ذكر/مكرر) لتقدير صفات الذبيحة كما تم جمع ١٢ عينة دم من طيور كل معاملة وقت الذبح لتقدير بعض مكونات سيرم الدم كما تم أيضا إجراء تجربة هضم فى نهاية فترة النمو لتقدير معاملات هضم المركبات الغذائية باستخدام ٦ ديوك من كل معاملة. وفى فترة البياض (٦-٢٠ أسبوع) تم تسجيل عدد ووزن وكتلة البيض ومعدل إنتاج البيض والغذاء المأكول ومعدل تحويل الغذاء كما تم تكسير عدد ١٠ بيضات من كل معاملة فى نهاية فترة الـ ٩٠ يوم من إنتاج البيض لتقدير جودة البيض وكذا التحليل الكيماوى للبيض.

من وجهة النظر الغذائية والإقتصادية يمكن أن يستنتج من النتائج تحت ظروف التجربة الحالية أن إضافة حمض الماليك بمعدل ٠,٠٥٪ إلى علائق السمان الياباني المحتوية على مستويات تحت المثلى من البروتين والطاقة ساعدت فى تقليل أعداد الكائنات الحية الدقيقة الضارة الموجودة بأمعاء الطيور وهذا بدوره يحسن أداء ومناعة الطيور كما يخفف أيضا من الأعباء المالية على المزارع وبعبارة أخرى إذا استخدم حمض الماليك بطريقة صحيحة مع ظروف جيدة من التغذية والرعاية والأمان الحيوى فإن حمض الماليك يصبح أداة قوية فى المحافظة على صحة القناة الهضمية مما يحسن من أداء الطيور.