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Early Feeding Enriched by Vitamin C and Date Syrup Modifies the Productive and Physiological Traits and Duodenal Histology of Japanese Quails

Research Article

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ABSTRACT

The study aimed to evaluate the influence of early feeding in post-hatch with dietary vitamin C (VC) and date syrup (DS) on productive, physiological, and histological attributes of Japanese quails. A total of 800 one-day-old quails were reared for 6 weeks and assigned into 8 groups including late feeding groups which fed on the farm 24 hours post-hatch with no feed additive (NO) or with 5 g VC or 1 mL DS/kg of diet or with a mixture of 2.5 g VC + 0.5 mL DS (MI)/kg of diet. Besides, groups of early feeding which fed post hatch immediately with NO or with 5 g VC or 1 mL DS/kg of diet or with MI/kg of diet. Each group had 100 chicks with 5 replications each. A completely randomized design was adopted and the main effects of the feeding method × feed additive were arranged in 2 × 4 factorial approach. As compared with the main effect of late feeding, the results showed that early feeding increased ($P \leq 0.01$) final body weight, carcass yields, production efficiency factor, and protein and energy efficiency ratios with a reduction ($P \leq 0.01$) in feed efficiency and mortality. Also, early feeding augmented ($P \leq 0.01$) serum protein, glucose, vitamin C, and ferric-reducing ability of plasma with lowering ($P \leq 0.01$) of serum cholesterol, aspartate transaminase, alanine aminotransferase, creatinine, uric acid, malondialdehyde, hydroperoxide, and corticosterone and heterophils to lymphocytes ratio. Moreover, early feeding increased ($P \leq 0.01$) villus height/crypt depth and increased ($P \leq 0.05$) villus surface area and muscular layer thickness in the duodenum. The main effect of DS in diet or its mixture with VC seemed to have a significant influence on the aforementioned traits compared with individual using of VC. It was concluded that interactions between early feeding and DS alone or with VC added to the diet could change positively productive and physiological aspects and duodenal morphology of quails.

KEY WORDS date syrup, early feeding, Japanese quail, vitamin C.

INTRODUCTION

It is well known that the production of good-quality one-day-old chicks is considered the pivotal link between hatchery and farm. Delay in feed access of hatchlings in post-hatch time about 24-72 hours is more often practiced in some commercial hatcheries because of prolonged delays in shipment or large distances of chick's transportation. These negative effects lead to a lowering in growth performance, initial body weights and enzymatic development of the digestive system with an inappropriate consumption of nutritive yolk in chicks (Noy and Sklan, 1998; Uni *et al*. 1998; Willemsen *et al*. 2010). Also, delay in feed access might result in high mobilization of body reserves from

muscles and subcutaneous liver and fat to accelerate the maturation of the thermoregulatory system which mainly takes place in the first ten days of the post-hatching life that has repercussions on the final performance of chicks (Nichelmann and Tzschentke, 2002; Willemsen *et al*. 2010). Multiple reports have revealed that early access to feed and water for neonatal chicks could positively influence of productive profile by marked mechanisms involving immediate activation the utilization rate of yolk sac and powerful stimulation of the digestive tissues and intestinal absorption (Noy and Sklan, 1998; Sklan, 2003) with functional development of immune system (Dibner *et al*. 1998; Tamboli *et al*. 2017; Hollemans *et al*. 2021). Enhanced growth of digestive villi and intestinal mucosa by first access to feed plays a crucial role in increasing nutrient utilization and reabsorption capacity the yolk sac which reflect on increased final body weight and overall subsequent performance (Williams *et al*. 2020). Also, early feeding demonstrated its influence to reduce mortality, and improve livability (Uni and Ferket, 2004) and stimulating resistance against diseases (Simon *et al*. 2015).

Vitamin C (VC) or ascorbic acid is a water-soluble vitamin synthesized from a glucose molecule, characterized by its reducing function as an electron carrier for donation by two electrons which converted in turn to dehydro-L-ascorbic acid which is very important as a cofactor in redox and hydroxylation reactions such as, cytochrome P450-dependent hydroxylation that controlled in metabolic pathways in live organisms (Pardue and Thaxton, 1986; Whitehead and Keller, 2003; Shojadoost *et al*. 2021). Vitamin C can react with free radicals and scavenge them rapidly, participates to the generation of antioxidant properties in a biological system, and mitigates the negative impacts of stress (Sahin *et al*. 2004). Therefore, VC was routinely used as anti-stressor factor in poultry model because of its ability to alter immune system related genes transcription, and reduces proinflammatory cytokines with anti-inflammatory and other immunoregulation roles (Zhu *et al*. 2019; Shakeri *et al*. 2020; Shojadoost *et al*. 2021). This various mechanisms of VC could enhance productive efficiency, nutrient digestibility, fertility, semen quality and carcass quality and lower mortality during heat stress (Khan *et al*. 2012; Abidin and Khatoon, 2013) with improved intestinal histoarchitecture in the pulmonary hypertensive broilers (Moghaddam *et al*. 2009).

High energy requirements depend basically on cereal grains and fats supplements which must meet the poultry diets. In many parts of the world, other alternative sources of energy may be found at low cost. One of the countries like Iraq produces in abundance a high mass of date palm (*Phoenix dactylifera*) fruit and its by-products at favorably priced *per annum*. Among these available sources is by-products of date such as, date waste meal (Attia and Al-Harthi, 2015; Najafi *et al*. 2021), and date pits (Alyileili *et al*. 2020) which have been incorporated in poultry diet at different levels.

In addition, date syrup (DS) which is called dibs, is the most common natural product of date palm fruit which is obtained by concentrating the date juice. It is characterized by an antioxidant, anti-tumor, anticancer, anti-mutagenic and antibacterial activity because of its contents of polyphenols, carotenoids and flavonoids with very complex compounds of saccharides, calcium organic and amino acids (Ganbi, 2012; Abbès *et al*. 2013; Taleb *et al*. 2016). These phytochemicals in DS participate to nutritional and organoleptic properties with powerful health advantages (Abbès *et al*. 2013). On basis that reason, DS and whole palm date fruits as a source of energy were recommended to be consumed before and after delivery by pregnant women and for treatment of liver diseases in traditional medicine (El-Hamzy *et al*. 2013). Despite the multiple benefits and cheapness of DS, it was not highlighted for using in poultry diets as a feed additive so far. Therefore, the scarcity in published data on biological attributes of DS in poultry feeding is existent in the literature. Moreover, Japanese quail rearing received much less consideration globally because it is well-known that the commercial poultry industry is governed by broilers and turkeys. Hardly any information is obtainable concerning the impacts of early feeding in the post-hatch on the future life of quail and also impacts of DS mixed in the diet as compared with commercial vitamin C was not revealed on poultry species. On basis that hypotheses, the current study was designed to evaluate these effects on quail productivity and physiological, histological and antioxidative responses from hatching time until the marketing age.

MATERIALS AND METHODS

Experiment plan

The experiment was lasted for 6 weeks and conducted in Poultry Farm which belongs to the Technical College of Al-Musaib, Al-Furat Al-Awsat Technical University, Babylon, Iraq. In total, 800 one-day-old unsexed Japanese quail chicks with an initial body weight (6.18 g) were purchased from local hatchery (Babylon, Iraq). The chicks were individually weighed and distributed randomly into 2 basic groups, the 1st group was lately fed 24 hours post-hatch on the farm (late feeding) whereas 2nd group was immediately fed post-hatch in the hatchery (early feeding). The 1st basic group was assigned to 1st subgroup which involved chicks that lately fed after 24 hours post-hatch with no feed additive (NO), whereas 2nd, 3rd and 4th subgroups were subjected to the late feeding accompanied by offering 5 g/kg

vitamin C (VC) or 1 mL date syrup (DS)/kg of diet or mixture of 2.5 g VC and 0.5 mL DS (MI)/kg of diet. The 2nd basic group was assigned into 5th subgroup which involved chicks that fed directly post hatch with no feed additive (NO), while the latter subgroups (6th, 7th and 8th) were subjected to early feeding with offering 5g VC or 1 mL DS/kg of diet or mixture of 2.5 g VC and 0.5 mL DS (MI)/kg. Each group contained 5 replicate cages and 20 birds per replicate.

Animals' management and ethical issues

The chicks were reared in an environmentally controlled room divided into 40 wire cage system with the available provision of 24 hours lighting schedule. The temperature was kept at 35 ˚C with 65% of relative humidity for the initial 2 days of rearing and then the temperature and relative humidity were decreased to 21 ˚C and 45%, respectively until the end of the experiment. All of the procedures used in the experiment were carried out according to guidelines regarding to animal welfare approved by the Scientific Committee of the Department of Animal Production Techniques, Al-Furat Al-Awsat Technical University (Babylon, Iraq). Birds were fed and watered *ad libitum* with an isonitrogenous and isocaloric diet based on yellow corn and soybean meal (NRC, 1994; Table 1).

Feed additives

Vitamin C (80%- Inner Mongolia HuaTian Pharmaceutical Co., Ltd, China) was obtained from local market and was in soluble powder form as a feed additive for poultry. Date syrup (Karbala for Food Canning Co. Ltd, Iraq) was in the natural product (100%) with no additives and industrial preservatives licensed by the Iraqi Ministry of Health with nutritive value containing 76.3% total solid, 23.7% water, 75% carbohydrates, 35.5% glucose, 27.9% fructose, 2.5% protein, 8% fiber and 0.4% fat with 2.2% of mineral salts including K, Ca, Mg, P, S, Mn and Fe and vitamins (A, B1, and C). Moreover, DS has total phenolic compounds which are expressed as 180 mg of gallic acid equivalent per 100 g and 86.5 µg of total carotenoids per 1 g.

The additives were appropriately mixed with the homogenized way in feed by taking a small amount of each additive and mix thoroughly with 1 kg of feed and a thereafter small amount from this prepared mixture was mixed into more quantities of feed gradually according to the determined dose and served to birds daily until 42 days.

Characteristics studied

Growth performance

Productive characteristics encompassed body weight (BW), feed intake (FI), feed conversion ratio (FCR), the protein efficiency ratio (PER), the energy efficiency ratio (EER), and mortality was recorded weekly for each replicate in group and presented cumulatively from 1 week to 3 and 6 weeks. Production efficiency factor (PEF) as a profit indicator of the experiment was calculated according to the following formula (final BW, kg×livability, %) / (marketing age, days×FCR×100) which was coined by Lemme *et al*. (2006). A total 160 sexed birds (1 male and 3 females birds/replicate, 20 birds/group) were selected randomly and slaughtered at 6 weeks old to evaluate the carcass properties involving carcass yield with or without giblets (heart, liver, and gizzard) and each carcass cut (breast, thighs, wings, and trunk) and body fat content was calculated proportionally to carcass weight. All birds were maintained for 6 hours under a fasting schedule to evacuate their digestive system and stabilize their final weights before had been slaughtered.

Duodenal histomorphological changes

The protocol of Rubio *et al*. (2010) was followed to calculate the histological analysis of villus height (VH), villus width (VW), villus surface area (VSA), crypt depth (CD), villus height/crypt depth (VH/CD) and muscular layer thickness (MLT) which can be summarized by dissect 1 cm of duodenum section of the small intestine which fixated immediately in 10% buffered neutral formalin and exposed to hydration in ethanol, clearance in xylene, and embedding with paraffin wax. Subsequently, a 4 µm thickness of duodenal segment was sliced using a microtome (Leica RM 2155, England) and stained with hematoxylin and eosin for microscopical investigation from 1 male and 2 females per replicate of group with 2 sections per bird and 10 readings (slides) per bird (n=20 slides per bird in replicate).

Biochemical metabolites and stress indicators

In 6th week, blood samples were collected after slaughtering from the jugular vein of 1 male and 2 female birds/replicate, 15 birds/group. The first collection of blood was placed in tubes containing K_3EDTA as an anticoagulant to obtain plasma or for the count heterophils to lymphocytes ratio (H/L) (Burton and Guion, 1968). The second collection of blood was placed in a serum separator gel tube without anticoagulant which was left at room temperature to clot and then centrifuged at 1500 rpm for 15 min to separate serum which was preserved in deep freezing at -20 ˚C until biochemical analysis. The biochemical metabolites in serum were spectrophotometrically determined using a visible spectrophotometer VS721G (Yucheng Tech, China) and commercial diagnostic bioassay kits (Biolabo, French) were used to determine total protein, cholesterol (Young, 2000), creatinine and uric acid (Tietz, 1999). Glucose level was calculated based on the method reported by Young (2000) using Cromatest kit (Spanish).

Table 1 Diet composition and chemical content of nutrients

Ingredients	(%)	Calculated analysis**	
Yellow corn	53.03	Crude protein (%)	24.0
Soybean meal	38.69	Metabolizable energy (kcal/kg)	2901
Corn gluten	3.200	Calcium (%)	0.83
Sunflower oil	1.670	Available phosphorus (%)	0.42
Dicalcium phosphate	0.810	Methionine (%)	0.50
Limestone	0.300	Methionine + cysteine (%)	0.80
Premix*	0.300	Lysine (%)	1.59
NaCl	0.110	Calcium (%)	0.80
DL-methionine	0.390		
L-lysine	1.500	Crude fiber (%)	3.90
Total	100.00		

* Provimi premix 3110 (Jordan) provided per kilogram of diet, Metabolizable energy: 3800 kcal; Crude protein: 7%; Fat: 1.1%; Lysine: 4%; Phosphorus: 11%; Sodium: 4.8%; Calcium: 5.4%; Methionine: 8.5%; Methionine + cysteine: 8.5%; Threonine: 0.55%; vitamin A: 57500 IU; vitamin D_3: 20125 IU; vitamin E: 3000 mg; vitamin K_3: 138 mg; vitamin B_1: 138 mg; vitamin B_2: 345 mg; vitamin B_3: 1840 mg; vitamin B_5: 552 mg; vitamin B_6: 184 mg; vitamin B_9: 46 mg; vitamin B_{12}: 1000 mg; Biotin: 6900 g; Choline chloride: 20000 mg; Iron: 2760 mg; Zinc: 3680 mg; Manganese: 3680 mg; Selenium: 9.2 mg and Iodine: 50 mg.
** According to NRC (1994).

For the determination of alanine aminotransferase (ALT) and aspartate transaminase (AST) activity, a Randox enzymatic test kit (English) was used according to Reitman and Frankel (1957). The ferric-reducing ability of plasma (FRAP) was spectrophotometrically estimated based on an analytical method coined by Benzie and Strain (1996). Regarding the biomarker of lipid peroxidation which included malondialdehyde (MDA) and hydroperoxide (LOOH), the methods of Salih *et al*. (1987) and Södergren *et al*. (1998) were followed for determination, respectively using a commercial kit (Sigma Aldrich, St. Louis, MO, USA). Corticosterone hormone in serum was analyzed using a specific enzyme-radioimmunoassay kit (IDS, Boldon, UK) by following the described steps of manufacturer recommendations and using ELISA microplate reader (MRX® II Dynex Technologies, USA) to measure the absorbance. The colorimetric method of vitamin C in blood serum was spectrophotometrically determined (Kyaw, 1978) by using an assay kit (Sigma-Aldrich, USA).

Statistical analysis

To find the influence of the main effectof feeding method factor (early feeding and late feeding)and main effectof feed additives factor (no feed additive; NO, vitamin C; VC, date syrup; DS and mixture of VC and DS; MI) and interactive treatments (2×4) on variable studied, a completely randomized design was used in analyzing the data by using the software program SAS (2012). The Duncan test (Duncan, 1955) was implemented to compare the significant differences among group means and to indicate significant differences on probability level ($P<0.05$) and ($P<0.01$) based on statistical model:

$$Y_{ijk}= \mu + FM_i + FA_j + (FM\times FA)_{ij} + e_{ijk}$$

Where:

Y_{ijk}: variable under influence of treatment.
μ: general average of the variable.
FM_i: influence of feeding method (i=2).
FA_j: influence of feed additive (j=4).
$(FM\times FA)_{ij}$: interaction between feeding method and feed additive (8 treatments).
e_{ijk}: random error.

RESULTS AND DISCUSSION

The results presented in Table 2 show that BW, FI, PER, and EER were higher significant ($P\leq0.05$) in interactive treatments (early feeding with NO, VC, DS and MI) followed by (late feeding with VC, DS, and MI) than late feeding with NO from 1-3 and 1- 6 weeks. Also, mortality and FCR in the same groups were reduced ($P\leq0.05$) compared with late feeding with NO. The early feeding registered highly significant values ($P\leq0.01$) in final BW, FI (1-3 weeks), PER (1-6 weeks), and EER with reduction ($P\leq0.01$) in FCR and mortality compared with late feeding. In comparison to NO, it was an increase ($P\leq0.05$) in BW for DS and MI at 6 weeks. Also, an increase ($P\leq0.05$)was recorded at 1-6 weeks in FI for MI and in EER for VC and MI. Low ($P\leq0.05$) FCR and mortality were in VC, DS, and MI compared with NO. Table 3 revealed that a high significant increase ($P\leq0.01$) in carcass yield without giblets and carcass yield with giblets was obtained in (early feeding with VC, DS, and MI , late feeding with VC and DS) and (early feeding with VC, DS and MI and late feeding with VC, DS and MI), respectively compared with late feeding with NO. In comparison to late feeding with NO, it was a high ($P\leq0.05$) proportional weight of breast for late feeding with MI, early feeding with NO, VC, DS, and MI. Low ($P\leq0.05$) body fat and high PEF for all interactive groups compared with late feeding with NO with a lack of significant differences among groups in other traits.

Table 2 Productive performance of Japanese quail chicks influenced by feeding method with dietary vitamin C and date syrup

Treatment	Week	BW (g)		FI (g)		FCR (g/g)		PER (g/g)		EER (g 100/kcal)		Mortality (%)
		3	6	1-3	1-6	1-3	1-6	1-3	1-6	1-3	1-6	1-6
Feeding method	Feed additive	Feeding method × feed additive										
Late feeding	NO	71.35^{c}	139.28^{c}	170.84^{c}	590.53^{b}	2.62^{a}	4.48^{a}	1.59^{b}	0.92^{b}	13.15^{b}	7.691^{b}	10.0^{a}
	VC	75.16^{b}	147.54^{b}	171.45^{bc}	610.32^{a}	2.48^{ab}	4.31^{a}	1.67^{a}	0.96^{a}	13.86^{ab}	7.984^{b}	7.00^{ab}
	DS	74.22^{b}	148.87^{ab}	170.33^{c}	615.63^{a}	2.50^{a}	4.31^{a}	1.66^{ab}	0.96^{a}	13.77^{b}	7.989^{b}	5.50^{b}
	MI	76.13^{ab}	147.49^{b}	174.47^{a}	603.36^{a}	2.49^{b}	4.26^{ab}	1.67^{a}	0.97^{a}	13.82^{b}	8.073^{ab}	6.00^{b}
Early feeding	NO	73.25^{b}	145.85^{b}	175.47^{a}	596.34^{ab}	2.60^{a}	4.37^{a}	1.59^{b}	0.95^{a}	13.17^{b}	7.875^{b}	4.00^{bc}
	VC	77.10^{a}	149.36^{ab}	173.66^{ab}	611.33^{a}	2.46^{b}	4.12^{b}	1.69^{a}	1.01^{a}	13.98^{a}	8.360^{a}	2.00^{c}
	DS	79.89^{a}	151.27^{a}	175.25^{a}	600.35^{a}	2.38^{c}	4.13^{b}	1.74^{a}	1.00^{a}	14.46^{a}	8.330^{a}	0.00^{d}
	MI	80.40^{a}	156.43^{a}	176.54^{a}	619.65^{a}	2.38^{c}	4.12^{b}	1.75^{a}	1.01^{a}	14.48^{a}	8.358^{a}	0.00^{d}
	SEM	3.75	2.98	2.87	1.87	0.33	0.45	0.14	0.12	3.21	3.82	0.97
	P-value	0.041	0.022	0.040	0.032	0.021	0.047	0.026	0.043	0.025	0.030	0.028
		Feeding method										
Late feeding		74.22^{B}	145.63^{B}	171.5^{B}	606.46	2.62^{A}	4.44^{A}	1.65	0.95^{B}	13.22^{B}	7.83^{B}	7.0^{A}
Early feeding		78.18^{A}	151.32^{A}	176.2^{A}	605.41	2.41^{B}	4.19^{B}	1.69	1.10^{A}	14.32^{A}	8.63^{A}	1.5^{B}
	SEM	0.65	0.83	2.87	2.85	0.46	0.62	0.16	0.13	4.75	3.98	0.16
	P-value	0.010	0.014	0.012	0.140	0.012	0.011	0.129	0.014	0.012	0.003	0.000
		Feed additive										
NO		72.31^{b}	142.56^{b}	173.15^{ab}	603.93^{b}	2.61^{a}	4.52^{a}	1.59^{b}	0.94	13.65^{b}	7.78^{b}	7.0^{a}
VC		75.87^{a}	148.45^{ab}	172.55^{b}	600.33^{b}	2.47^{b}	4.22^{b}	1.68^{ab}	0.98	13.92^{ab}	8.17^{a}	4.5^{b}
DS		77.00^{a}	150.07^{a}	172.79^{b}	607.99^{ab}	2.44^{b}	4.22^{b}	1.70^{a}	0.98	14.12^{a}	8.16^{ab}	2.5^{c}
MI		78.24^{a}	151.96^{a}	175.50^{a}	611.50^{a}	2.43^{b}	4.19^{b}	1.71^{a}	0.99	14.15^{a}	8.21^{a}	3.0^{bc}
	SEM	4.43	5.73	3.32	1.44	0.72	0.73	0.24	0.16	3.53	4.36	0.28
	P-value	0.050	0.040	0.037	0.029	0.036	0.025	0.049	0.096	0.052	0.041	0.046

BW: body weight; FI: feed intake; FCR: feed conversion ratio; PER: protein efficiency ratio; EER: energy efficiency ratio; NO: no feed additive; VC: vitamin C (5 g/kg of diet); DS: date syrup (1 mL/kg of diet) and MI: a mixture of vitamin C and date syrup (2.5 g+0.5 mL/kg of diet, respectively).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Carcass quality and production efficiency factor of Japanese quail chicks influenced by feeding method with dietary vitamin C and date syrup

Treatment		Carcass yield (%)		Breast (%)	Thighs (%)	Wings (%)	Trunk (%)	Body fat (%)	PEF
		Without giblets	With giblets						
Feeding method	Feed additive	Feeding method × feed additive							
Late feeding	NO	65.32^{B}	70.28^{C}	33.65^{c}	23.44	11.95	30.44	1.97^{a}	6.65^{c}
	VC	67.30^{A}	72.63^{B}	33.64^{c}	23.63	12.75	29.57	0.79^{b}	7.56^{b}
	DS	67.62^{A}	72.86^{B}	33.32^{c}	23.74	12.43	30.25	0.88^{b}	7.80^{ab}
	MI	66.89^{AB}	72.11^{B}	34.54^{b}	23.43	12.44	29.46	0.89^{b}	7.73^{b}
Early feeding	NO	66.33^{B}	71.73^{BC}	34.34^{b}	24.35	11.79	29.55	1.07^{a}	7.61^{b}
	VC	67.83^{A}	73.46^{A}	35.43^{a}	23.64	11.44	29.33	0.86^{b}	8.45^{a}
	DS	68.32^{A}	74.19^{A}	35.47^{a}	24.14	11.15	29.25	0.87^{b}	8.70^{a}
	MI	67.38^{A}	72.88^{AB}	34.17^{b}	24.36	11.24	30.57	0.75^{b}	9.03^{a}
	SEM	4.98	2.98	5.09	6.76	4.93	6.98	0.09	0.76
	P-value	0.013	0.010	0.047	0.212	0.423	0.634	0.052	0.049
		Feeding method							
Late feeding		66.28^{B}	71.17^{B}	33.78	23.56	12.39	29.93	1.13	7.14^{B}
Early feeding		68.10^{A}	73.56^{A}	34.85	24.12	11.41	29.67	0.88	8.95^{A}
	SEM	6.42	3.61	4.12	5.23	3.52	5.73	0.07	0.86
	P-value	0.009	0.012	0.256	0.176	0.123	0.112	0.654	0.011
		Feed additive							
NO		65.82^{b}	71.00^{b}	33.99	23.89	11.87	29.99	1.52	7.13
VC		67.56^{a}	73.04^{a}	34.53	23.63	12.09	29.45	0.82	8.00
DS		67.97^{a}	73.52^{a}	34.39	23.94	11.79	29.75	0.87	8.25
MI		67.13^{ab}	72.49^{ab}	34.35	23.89	11.84	30.01	0.82	8.38
	SEM	8.78	6.35	4.49	6.34	3.44	7.32	0.08	0.73
	P-value	0.029	0.038	0.984	0.432	0.265	0.878	0.765	0.542

PEF: production efficiency factor; NO: no feed additive; VC: vitamin C (5 g/kg of diet); DS: date syrup (1 mL/kg of diet) and MI: a mixture of vitamin C and date syrup (2.5 g+0.5 mL/kg of diet, respectively).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

It was high (P≤0.05) carcass yields and PEF (P≤0.01) in early feeding compared to late feeding. VC and DS had high (P≤0.05) carcass yields compared to NO with no obvious differences among groups in other parameters.

Based on the results shown in Table 4 it is visible that high (P≤0.05) protein levels in blood serum were achieved by early feeding with NO, VC, DS, and MI and late feeding with VC and MI. High (P≤0.05) glucose level was obtained by early feeding with NO, DS and MI and late feeding with VC, DS and MI with lowering (P≤0.05) of cholesterol, creatinine and uric acid levels in all groups compared with late feeding with NO. Moreover, notable decrease (P≤0.05) in ALT for early feeding with NO, VC, DS and MI and late feeding with DS and MI whereas low AST was in early feeding with VC, DS and MI and late feeding with DS compared with late feeding NO. Early feeding improved (P≤0.01) all serum metabolite levels compared with late feeding. All feeding additives increased (P≤0.05) glucose, decreased (P≤0.05) cholesterol, and decreased (P≤0.01) AST, creatinine and uric acid whereas high (P≤0.05) glucose was obtained by VC and MI and low (P≤0.05) ALT was in DS and MI.

In the results of Table 5 it was lowering (P≤0.05) in H/L for all interactive groups except for late feeding with DS compared with late feeding with NO. Also, high values (P≤0.05) in FRAP and vitamin C with a low value in MDA and LOOH were in all groups whereas the corticosterone level was only decreased (P≤0.05)in early feeding with VC and MI and late feeding with VC in comparison to late feeding with NO. Early feeding registered low averages (P≤0.01) in H/L, MDA, LOOH, and corticosterone with increasing values (P≤0.01) in FRAP and vitamin C compared with late feeding. The minimal levels in H/L and MDA (P≤0.01) and corticosterone (P≤0.05) and high levels (P≤0.01) in vitamin C and FRAP were in VC, DS and MI compared with NO whereas a decrease (P≤0.01) in LOOH was in favor of DS and MI.

High (P≤0.05) VH, VSA and MLT were recorded for all interactions compared with late feeding with NO. Also, it was increased values (P≤0.05) of VW, CD and VH/CD in the most of interactions compared with late feeding with NO. In comparison to late feeding, early feeding had high averages (P≤0.05) in VH, VW, VSA and MLT with an increase (P≤0.01) VH/CD. All feed additives groups increased (P≤0.05) VH, VSA and MLT whereas a noticeable increasing (P≤0.05) in VW and CD was only in favor of DS and MI with high value (P≤0.05) in VH/CD was for VC and MI compared to NO (Table 6).

Improved overall productive and physiological performance of chicks under the main effect of early feeding group or interactions between early feeding with VC, DS and MI which observed in current results might belong to role of first access to feed for supporting metabolism, thermal regulation, increased intestinal motility and improved BW through decrease mobilization of body reserves with saving energy and muscle protein synthesis (Uni and Ferket, 2004; Willemsen *et al*. 2010; Williams *et al*. 2020). This could be resulted by positive modifications in the digestive system which occur in future life such as, multiple enlargements of the absorptive surface of the intestinal villi, prevention of clumping in microvilli and differentiation of enterocytes and crypt depth. Also, activation the digestibility by active stimulation of the brush border enzyme, fast utilization of yolk materials, and enhancing biliary pancreatic secretions was proved by early feeding (Noy and Sklan, 1998; Uni *et al*. 1998).

These crucial changes are sensitive to delay in nutrients offering and are clearly dependant upon yolk materials and early access to feed. Also, in current data, delay in feed access might cause future exacerbation in lowering FI due to a reduction in some metabolic hormones such as, triiodothyronine (T3) level which is linearly correlated with FI and it stimulates cell proliferation, basal metabolic rate, and oxidative metabolism (Noy *et al*. 2001; De Jong *et al*. 2017). Moreover, the impaired general growth rate and BW in feed-deprived chicks in our findings might attribute to poor utilization and less retention of nutrients represented by reducing nitrogen-corrected metabolizable energy (Corless and Sell, 1999) and adversely enhances nonesterified fatty acid with higher use of fatty acids in plasma for energy utilization (Noy *et al*. 2001). These evidence were in line with the amelioration the biochemical profile of liver and kidney (Table 4) and the improvement of duodenal histomorphology in current data (Table 6). Also, it can be reasoned that first provision to feed could increase glucose content which is necessary as the primary source of energy in hatching chicks because there is a considerable reduction in muscular glycogen during hatching event required for post-hatch life (Prabakar *et al*. 2016).

Researches with broiler chickens declared that early access to feed enhanced immune system development and disease resistance. For instance, Simon *et al*. (2015) found that feeding chicks immediately post hatch could be less sensitive to intratracheal challenge by a combination of 2.5 mg/kg of *Escherichia coli* lipopolysaccharide and 0.5 mg/kg of human serum albumin which resulted the lowest antibody titers against immune challenges compared to groups fed after 72 h post-hatch. Also, it was concluded that cell-mediated immune response, Newcastle disease titers and weight of bursa improved at 35 days of age in chicks fed by intubation into the crop immediately post-hatch with 0.8 mL starch individually, with 0.6 mL casein or with 0.4 mL soybean oil compared to starved chicks for 24 hours post-hatch (Bhanja *et al*. 2010).

Table 4 Levels of biochemical metabolites in blood serum of Japanese quail chicks influenced by feeding method with dietary vitamin C and date syrup

Treatment		Protein (g/dL)	Glucose (mg/dL)	Cholesterol (mg/dL)	ALT (U/L)	AST (U/L)	Creatinine (mg/dL)	Uric acid (mg/dL)
Feeding method	Feed additive	Feeding method × feed additive						
Late feeding	NO	3.12^{c}	200.44^{c}	184.16^{a}	36.82^{a}	163.94^{a}	1.98^{a}	5.27^{a}
	VC	3.53^{b}	226.82^{ab}	168.94^{bc}	33.53^{ab}	158.35^{ab}	1.32^{bc}	4.38^{b}
	DS	3.23^{bc}	223.36^{b}	174.11^{b}	32.42^{b}	154.54^{c}	1.32^{bc}	4.32^{b}
	MI	3.64^{b}	222.53^{b}	173.59^{b}	33.38^{b}	158.25^{ab}	1.48^{b}	4.34^{b}
Early feeding	NO	4.48^{a}	228.67^{a}	170.65^{b}	23.46^{c}	158.53^{ab}	1.42^{b}	4.32^{b}
	VC	4.77^{a}	220.33^{bc}	162.23^{c}	28.34^{c}	126.26^{d}	1.34^{b}	4.17^{b}
	DS	4.86^{a}	230.92^{a}	161.74^{d}	26.42^{c}	143.74^{cd}	1.18^{c}	4.12^{b}
	MI	4.84^{a}	228.22^{a}	167.28^{c}	24.43^{c}	136.76^{d}	1.24^{c}	4.16^{b}
	SEM	0.94	24.98	11.98	1.98	21.98	0.21	1.98
	P-value	0.020	0.032	0.050	0.043	0.023	0.036	0.047
		Feeding method						
Late feeding		3.38^{B}	218.28^{B}	184.16^{A}	34.03^{A}	158.77^{A}	1.52^{A}	4.57^{A}
Early feeding		4.73^{A}	227.03^{A}	165.47^{B}	25.66^{B}	141.32^{B}	1.29^{B}	4.19^{B}
	SEM	0.87	32.98	15.98	2.98	28.09	0.13	1.65
	P-value	0.000	0.010	0.010	0.000	0.010	0.000	0.013
		Feed additive						
NO		3.80^{b}	214.55^{b}	177.40^{a}	30.93^{A}	161.23^{A}	1.70^{A}	4.89^{A}
VC		4.20^{a}	223.57^{a}	173.19^{b}	30.14^{AB}	142.30^{C}	1.33^{B}	4.27^{B}
DS		4.04^{ab}	227.14^{a}	172.95^{c}	29.42^{B}	149.14^{B}	1.25^{C}	4.22^{B}
MI		4.24^{a}	225.37^{a}	175.72^{b}	28.90^{B}	147.50^{B}	1.36^{BC}	4.25^{B}
	SEM	0.87	27.37	18.98	3.05	23.98	0.26	1.34
	P-value	0.036	0.042	0.028	0.008	0.011	0.012	0.009

ALT: alanine aminotransferase; AST: aspartate transaminase; NO: no feed additive; VC: vitamin C (5 g/kg of diet); DS: date syrup (1 mL/kg of diet) and MI: a mixture of vitamin C and date syrup (2.5 g+0.5 mL/kg of diet, respectively).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Levels of stress indicators in blood of Japanese quail chicks influenced by feeding method with dietary vitamin C and date syrup

Treatment		H/L	FRAP (µmol/L)	MDA (µmol/L)	LOOH (µmol/L)	Vitamin C (Mmol/L)	Corticosterone (µg/dL)
Feeding method	Feed additive	Feeding method × feed additive					
Late feeding	NO	0.30^{a}	113.14^{d}	1.52^{a}	23.52^{a}	44.34^{d}	0.15^{a}
	VC	0.23^{bc}	123.32^{b}	0.74^{c}	19.37^{b}	57.81^{a}	0.04^{b}
	DS	0.25^{ab}	122.32^{b}	0.84^{b}	19.32^{b}	52.48^{b}	0.06^{ab}
	MI	0.24^{b}	123.63^{ab}	0.83^{b}	20.76^{b}	56.74^{a}	0.05^{ab}
Early feeding	NO	0.24^{b}	119.15^{c}	0.89^{b}	20.12^{b}	49.14^{c}	0.08^{ab}
	VC	0.23^{bc}	125.16^{a}	0.49^{d}	19.41^{b}	63.93^{a}	0.04^{b}
	DS	0.22^{c}	124.12^{a}	0.55^{d}	18.36^{bc}	54.87^{ab}	0.05^{ab}
	MI	0.23^{bc}	127.63^{a}	0.54^{d}	17.43^{c}	62.14^{a}	0.04^{b}
	SEM	0.02	5.98	0.01	1.87	8.87	0.03
	P-value	0.027	0.036	0.049	0.040	0.029	0.038
		Feeding method					
Late feeding		0.26^{A}	120.62^{B}	0.98^{A}	20.84^{A}	52.36^{B}	0.07^{A}
Early feeding		0.23^{B}	123.89^{A}	0.61^{B}	18.73^{B}	57.72^{A}	0.05^{B}
	SEM	0.02	4.28	0.03	1.98	7.29	0.02
	P-value	0.006	0.002	0.001	0.004	0.010	0.012
		Feed additive					
NO		0.27^{A}	115.94^{B}	1.20^{A}	21.82^{A}	46.99^{C}	0.11^{a}
VC		0.23^{B}	124.24^{A}	0.61^{B}	19.39^{AB}	60.87^{A}	0.04^{b}
DS		0.23^{B}	123.22^{A}	0.69^{B}	18.84^{B}	53.67^{B}	0.05^{b}
MI		0.23^{B}	125.63^{A}	0.68^{B}	19.09^{B}	59.44^{A}	0.04^{b}
	SEM	0.01	6.98	0.01	0.99	4.98	0.03
	P-value	0.005	0.012	0.013	0.002	0.011	0.040

H/L: heterophils to lymphocytes ratio; FRAP: ferric-reducing ability of plasma; MDA: malondialdehyde; LOOH: hydroperoxide; NO: no feed additive; VC: vitamin C (5 g/kg of diet); DS: date syrup (1 mL/kg of diet) and MI: a mixture of vitamin C and date syrup (2.5 g+0.5 mL/kg of diet, respectively).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Duodenal histomorphology of Japanese quail chicks influenced by feeding method with dietary vitamin C and date syrup

Treatment		VH (µm)	VW (µm)	CD (µm)	VH/ CD	VSA (×10³µm²)	MLT (µm)
Feeding method	Feed additive	Feeding method × feed additive					
Late feeding	NO	427.32^{c}	84.43^{b}	73.24^{c}	5.73^{c}	113.43^{d}	44.22^{c}
	VC	532.63^{a}	89.54^{a}	77.46^{a}	6.88^{b}	149.82^{b}	49.23^{b}
	DS	495.43^{ab}	89.62^{a}	78.57^{a}	6.30^{bc}	139.88^{c}	48.26^{b}
	MI	518.57^{a}	87.87^{ab}	76.83^{ab}	6.74^{b}	143.15^{b}	51.27^{a}
Early feeding	NO	479.77^{b}	86.36^{b}	74.37^{bc}	6.54^{b}	129.99^{c}	47.97^{b}
	VC	584.33^{a}	89.98^{a}	75.64^{b}	7.84^{a}	165.27^{a}	54.47^{a}
	DS	523.53^{a}	96.28^{a}	79.78^{a}	6.67^{b}	158.35^{a}	64.45^{a}
	MI	596.74^{a}	94.85^{a}	78.44^{a}	7.63^{a}	177.81^{a}	62.46^{a}
	SEM	48.98	4.98	3.09	0.94	59.98	2.98
	P-value	0.037	0.042	0.038	0.050	0.028	0.035
		Feeding method					
Late feeding		493.56^{b}	87.46^{b}	76.53	6.14^{B}	136.47^{b}	48.24^{b}
Early feeding		545.84^{a}	91.86^{a}	76.86	7.99^{A}	157.81^{a}	57.93^{a}
	SEM	59.20	8.93	6.76	0.89	49.43	8.98
	P-value	0.047	0.025	0.038	0.001	0.027	0.043
		Feed additive					
NO		453.19^{c}	85.39^{b}	73.75^{b}	6.14^{c}	121.65^{b}	45.89^{b}
VC		558.48^{a}	89.76^{ab}	76.42^{ab}	7.31^{a}	157.50^{a}	51.95^{a}
DS		509.48^{b}	92.95^{a}	79.12^{a}	6.43^{bc}	148.92^{a}	56.35^{a}
MI		557.65^{a}	91.36^{a}	77.48^{a}	7.19^{ab}	160.48^{a}	56.86^{a}
	SEM	39.98	9.17	9.43	0.99	57.93	7.39
	P-value	0.036	0.050	0.041	0.043	0.021	0.051

VH: villus height; VW: villus width; CD: crypt depth; VH/CD: villus height/crypt depth; VSA: villus surface area; MLT: muscular layer thickness; NO: no feed additive; VC: vitamin C (5 g/kg of diet); DS: date syrup (1 mL/kg of diet) and MI: a mixture of vitamin C and date syrup (2.5 g+0.5 mL/kg of diet, respectively).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

This might be a reasonable explanation for decrease of H/Land improve serum antioxidant status defense (Table 5) and decreased the total mortality. Differently, Kang *et al.* (2018) stated that no changes in the biochemical profile of broiler chickens' serum at 35 days involving triglyceride, protein, cholesterol, AST and differential count of leucocytes at 7, 21 and 35 days under the effect of early feeding in different times post hatch (3, 12, 24, 36 and 48 h), however, ALT was higher in chicks fed 36 and 48 h post-hatching. Also, different results were indicated by Noy *et al.* (2001) that serum glucose, triglycerides and phospholipids levels were not influenced by feed deprivation for 48 and 72 h in poults but there was decreasing in BW of the same birds compared with early fed birds. Our results are similar to that extensive study which included meta-analysis for the positive effect of post hatch feeding on productive and physiological performance of meat type and layer chickens and turkey poults (De Jong *et al.* 2017). The same researchers mentioned that delay access to feed and water up to 24 hours (12-36 hours) or up to 48 hours (36-60 hours) could depress BW, FI, development of gut segments with increased FCR, and total mortality.

Apparent improvement to a group of birds fed DS could attribute to nutritive elements of DS involving different levels of minerals and vitamins with bioactive compounds represented by total phenols, gallic acid, and carotenoids which are characterized by multiple properties responsible for the prevention of damaging influence of free radicals (Surai, 2014). Also, DS is considered as a source of high energy due it is content of 75% carbohydrates (35.5% of it is glucose share) as described in this current natural product. Perhaps not surprisingly, the carbohydrates are the important elements in an exogenous diet which are easily to degraded and ingested after endogenous transition from a lipid-rich yolk at early stage of chick life. This was also obvious to increase the blood antioxidant defense mechanism (FRAP and vitamin C) and reduce the stress markers (H/L, MDA, LOOH and corticosterone) (Table 5). Sugars and date by-products might possibly be more effectively to enhance hemopoiesis (Habibu *et al.* 2014), activating liver and renal functions and mitigate the influence deleterious of oxidative stress times (Najafi *et al.* 2021) in chickens. Thus, the antioxidant activity of DS product was via different pathways such as, binding of transition metal catalysts, inhibition of hydrogen atom abstraction, reductive capacity, suppression the chain propagation reactions, decomposition and scavenging of peroxides and other harmful free radicals (Abbès *et al.* 2013). Moreover, the polyphenols in DS and whole palm date fruit improve public health because of its antioxidative, hepatoprotective, anticarcinogenic, neuroprotective , anti-inflammatory, antiallergic characteristics and preventing cardiovascular diseases (Ganbi, 2012; El-Hamzy

et al. 2013). Besides, DS is powerful bacteriostatic and prooxidant to induce oxidative damage by generation H_2O_2 to suppress the growth of Gram-positive and Gram-negative *bacteria* such as, *Escherichia coli* and *Staphylococcus aureus,* respectively (Taleb *et al.* 2016). The sweet palatability of DS might be the main reason to increase FI in both late or early-fed chicks especially when mixed with VC (interaction) and increase final BW, improve FCR, carcass yield and other traits. Birds responded very quickly to alterations in dietary additives intake and this reflected on productive and physiological status. This would allow for the possibility to add DS in the diet without any physical or chemical limitations. Lack of carried-out researches respecting to using of DS in poultry diets with special reference to Japanese quail's diet was obvious. However, Kadhim *et al.* (2019) found that 1000 mL date molasses provided separately or synergistically with 50 g of VC per 1 L of drinking water could counteract the adverse effects of late feeding consequences (12 hours post hatch) of broiler chickens by improving BW, weight gain (WG), FI, water intake, FCR, carcass yield, PEF and lowering the mortality up to 6 weeks. Other sources of energy such as, molasses and crude sugars are available by-products of sugar production and have been used in poultry feeding because of their an low economical cost with no harmful side effects (Waldroup, 1981). For instance, the level of 1% trehalose of low-molecular-weight was found to be as motivation factor in chick's diet fed within 8 or 36 h of the hatch by obtaining high BW and FI at 21-day old (Bhuiyan *et al.* 2011). Unlike this research, Hussein *et al.* (2016) pointed out that the incorporation of 5 and 15% of sugar syrup in corn-soy basal diet did not affect WG, FI and FCR of broiler chickens at 34 days old. Also, different data from that were obtained by Abdelgader *et al.* (2019) who reported no differences in WG, FI, FCR and mortality at 4 weeks of broiler chickens fed dietary beet molasses at 5, 7.5 and 10%. In accordance with us, it was documented that the best FCR and PEF of broilers up to 40 days old were obtained with a diet containing date waste (source of energy) at 50 g/kg with increase serum total protein, albumin and globulin with low registered values of AST, ALT and creatinine (Attia and Al-Harthi, 2015). Also, an identical result was obtained recently by Najafi *et al.* (2021) that incorporated whole date waste by 10, 20, 30% and 40% in ostrich diet for 9 months alleviated the stress by reducing H/L, increasing serum glutathione peroxidase activity with high levels of lymphocyte and glucose to counteract the stress condition without negative effect on productivity or gut nutrients digestibility.

The main effect of industrial VC additives in overall present results was parallel or less powerful than effect of natural additives (DS) to modify the performance of hatched quails while immediately feeding post-hatch. The importance of VC individually or mixed with DS was proven in our data. This is principle indication to postulate the use of VC as a traditional anti stressor in poultry diet (Pardue and Thaxton, 1986) because of its scavenging activity of deleterious free radicals (Sahin *et al.* 2004) and endotoxins (Shakeri *et al.* 2020) with response modulation for humoral and cell mediated immune (Zhu *et al.* 2019). This would reflect in turn on controlling the inflammations and infectious and epidemic diseases by increasing the production of leukocytes, altering transcription of multiple immune-related genes in functional cells (Shojadoost *et al.* 2021) and reducing the incidence of mortality. The outcome of this study is in accordance with the many findings of potential VC in stressed Japanese quails. Mehmet *et al.* (2005) conveyed that 500 mg/kg of VC from 7 days until 4 weeks increased FI and WG with improved FCR although no notable effect was found in chilled carcass yield. The same was declared by Sahin *et al.* (2009) that 500 mg/kg of VC could increase FI and reduce the serum corticosterone at 135 d of layer Japanese quails exposed to thermal stress (34 ˚C) for 8 h per day. An increase in serum antioxidant status, decrease in corticosterone and H/L (Table 5), and increase liver and kidney functions (Table 4) in the present study, might have been due to the positive effects of VC by alleviating the negative effects of late feeding. This was probably because of activity of VC as antioxidants in specific pathways whereas it reacts and deactivates the aggressive radicals thus radical equivalents can be transferred to aqueous compartments from lipid phases (Abidin and Khatoon, 2013). This would be dependent upon to oxidation of VC to reversible form, dehydroascorbic acid, which is crucial in many biological roles or formation of acorbate radical by VC which attacks the single oxygen, hydroxyl, and superoxide radicals (Khan *et al.* 2012). Previous studies have shown that VC was associated with depressed stress and resulted in an increase in the total antioxidant capacity, superoxide dismutase, glutathione peroxidase and vitamin C levels with decreased malondialdehyde in plasma and tissues of quails (Sahin *et al.* 2003) or broiler chickens (El-Senousey, 2018). In compliance with Sigolo *et al.* (2019), was found that 1000 mg VC supplemented per kg of diet promoted quail health by lowering serum AST, ALT and cholesterol with elevating levels of protein and thyroid stimulating hormone which in turn led to the highest FI, final BW at 42 days old. These alterations might reflect on increased metabolism, digestibility and absorption which were represented by improved duodenal morphology that was higher with dietary VC, probably indicating a lowered response to late feeding stress with supplementation of this vitamin alone or in combination with DS. Similarly, Gan *et al.* (2020) reported that 500 mg/kg of VC increased BW

and decreased mortality over 35 days by improving intestinal morphology (VL, VL/CD), serum total antioxidant capacity, and immune properties with modification the structure of cecal microbiota for broiler chickens challenged by *Salmonella enteritidis*.

CONCLUSION

Early feeding post-hatch in the hatchery for quail chicks could improve future growth performance, feed efficiency, and intestinal histomorphology via alleviating the negative impacts of stress, ameliorating the blood biochemical indices compared with late feeding post-hatch 24 hours on the farm. Also, adding 1 ml natural DS per 1 kg diet individually or synergistically with 5 g VC per 1 kg diet coincided with early or late feeding proved its efficacy to improve productive and physiological attributes until 6 weeks old compared with added VC individually. Therefore, DS could be recommended as an alternative natural, cheap, and safe supplement with antistress properties in early feeding times compared to commercial, industrial, and high-priced VC.

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