IMPACT OF BETAINE, VITAMIN C AND FOLIC ACID SUPPLEMENTATIONS TO THE DIET ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF MATROUH POULTRY STRAIN UNDER EGYPTIAN SUMMER CONDITION

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Abstract: *The present study aimed to study the effects of betaine (1g/kg diet), vitamin C (200mg/kg diet) or folic acid (1mg/kg diet) supplementations either alone, together or in combinations to the diet on productive and reproductive traits, digestibility nutrient, and economic efficiency of Matrouh poultry strain from 24-36 weeks of age under Egyptian hot summer condition (from June to September2008).*

A total number of 240 laying hens and 48 cocks of Matrouh strain (local strain) were randomly distributed into eight equal groups (30 hens and 6 cocks/ each). Each group was nearly equal in average body weight. The averages minimum and maximum ambient temperatures ranged between 23.6 and 34.7 ^{0}C and relative humidity from 31.8 to 80.7%., during the experimental period.

Results obtained showed that the body weight at 32 or 36 weeks, feed conversion, egg production and egg mass were significantly ($P<0.05$) improved with the diet supplemented with betaine alone or in combination with vitamin C or folic acid as compared to control group.

Supplementation of betaine, vitamin C or folic acid together or in combinations significantly increased ($P < 0.05$) shell thickness, Haugh units, shell to egg weight, sperm motility, hemoglobin, total protein, globulin, antibody titer against SRBC'S and significantly decreased ($P < 0.05$) dead spermatozoa, serum glucose, cholesterol, HDL and tri-glycerdes as compared with control group. The digestion coefficient of crude protein and ether extract were significantly ($P<0.05$) increased with the diet supplemented with betaine alone or together with vitamin C or folic acid or in combinations as compared to the control group. The best economic efficiency was obtained with layers fed diet containing of betaine plus vitamin C and follow in combinations of betaine, vitamin C and folic acid as compared to other groups.

In conclusion, combinations of betaine (1g /kg diet), vitamin C (200mg/kg diet) and folic acid (1 mg /kg diet) improved the most productive and reproductive traits of Matrouh strain under Egyptian hot summer.

Key Words: Betaine, Vitamin C, Folic Acid, Productive, Reproductive, Matrouh.

INTRODUCTION

Nowadays in Egypt there is necessity of increasing animal production to fulfill the insisting demand of animal protein. It is noticed that the price of animal protein is getting higher during the last few years. So, the increase in animal protein production may come from the poultry. Poultry feeding and management could be considered very reasonable in cost if compared with the other animals. One of the problems challenging in poultry industry is the high ambient temperature, which persists for almost 5 months of the year (May to September) in Egypt, as it is can compromise the ability of birds to maintain homeostasis (Kadim *et al.*, 2008). It depresses body weight, egg production, weight, quality and hatchability and increase of mortality Pavlik *et al.* (2009). Heat stress begins when the ambient temperature becomes higher than 27°C and is readily apparent above 30°C (Bollengier-lee *et al.*, 1998). Various techniques are being practiced by the farmers to minimize the heat stress, such as adding antioxidant, vitamins or minerals such as betaine, vitamins C, E and folic acid, zinc and chromium (Sahin *et al.*, 2002).

Betaine has been well-established as a labile methyl donor and as an osmoprotectant, which is synthesized via the oxidation of betaine aldehyde formed by the irreversible oxidation of choline in the mitochondria of the liver and renal tissues (Ratriyanto *et al.*, 2009). Betaine is added to the feed of laying hens at a level of 600 ppm to significantly improve laying performance. So, it consequently contributes to enhanced productivity which could have an important economic value (Park *et al.*, 2006). The addition of betaine to the feeds in particular improves performance under stress conditions that affect cell osmolarity. Furthermore, betaine promotes intestinal microbes against osmotic variations and improves microbial fermentation activity, which in turn, may enhance nutrient digestibility (Ratriyanto *et al.*, 2009).

On the other hand, vitamin C is an indispensable micronutrient required to maintain the physiological processes of poultry (McDowell, 1989). Kutlu and Forbes (1993) reported that ascorbic acid reduces the synthesis of corticosteroid hormones in birds. Similarly, Sahin *et al.* (2002) reported that low concentrations of adrenocorticotropic hormone (ACTH) in quail reared at 32°C and fed a diet supplemented with vitamin C than in the heat-stressed controls. By decreasing synthesis and secretion of corticosteroids, vitamin C alleviates the negative effects of stress (McDowell, 1989). Desoky (2008) found that dietary supplementation of vitamin C (200 mg vitamin C/kg diet) or vitamin E (150 mg vitamin E/kg diet) alone or combined showed high ability of alleviating the negative effect of heat stress and improved egg production.

In addition, folic acid plays an important role in amino acid and DNA metabolism and its deficiency causes severe defects in DNA replication and repair (McDowell, 1989). Folic acid is also required for the methylation of homocysteine to form methionine (McDowell, 1989). Folic acid deficiency in growing of Japanese quail diet decreases live weight gain and feed efficiency, and increase of mortality, leg weakness and cervical paralysis (McDowell, 1989).

This study was carried out to establish dietary supplementation of betaine, vitamin C and folic acid, alone or together or in combinations on productive and reproductive traits, digestibility nutrient, and economic efficiency of Matrouh strain, under hot summer conditions.

MATERIALS AND METHODS

The experimental work of this study was carried out at Inshas Poultry Research Station, Animal Production Research

Institute, Egypt, during summer season (from June to September, 2008). The average minimum and maximum ambient temperatures ranged between 23.6 and 34.7 ^{0}C, relative humidity from 31.8 to 80.7% and temperature-humidity index (THI) from 21.64 to 33.52% under Inshas, Sharkia Governorate located in the eastern part of Nile Delta (30^{0} N), Egypt as show in Table 1. THI was estimated according to the formula by Marai *et al.* (2000) as follows:

THI=db^{0}C-{(0.31-0.31 RH) (db^{0}C - 14.4)}, where db^{0}C = bulb temperature in Celsius and RH= RH%/100.

A total number of 240 laying hens and 48 cocks of Matrouh local strain at 24 weeks of age were randomly distributed into 8 treatment groups (30 hens and 6 cocks / each treatment). All birds were housed individually in one cage. Each cage was provided with an individual feeder and one automatic pipette drinkers. The experimental groups were, nearly equal in average body weight (1413.50± 8.24 gm) in laying hens. The experimental period extended from 24 to 36 weeks of age. The experimental design is shown in Table (2). Birds were fed *ad-libitum* and fresh water was available all the time, during the experimental period. The photoperiod during the experimental period was fixed at 16h. The composition and chemical analysis of the experimental laying diet are presented in Table (3).

Individual body weight of laying hens was recorded at 24, 28, 32 and 36 weeks of age. While, egg number, egg weight and mortality rate were recorded daily and feed intake was calculated weekly. Egg mass was calculated by multiplying egg number by average egg weight. Feed conversions (g feed/g egg mass) were also calculated.

Monthly, 5 eggs from each treatment was broken at the same day to determine egg quality traits. Egg shape index (%) was estimated as the ratio of the egg maximum width to its length, shell thickness in mm. Arc-sine transformations were done for percentage of egg shape index and Haugh unit score was calculated for each individual egg according to Haugh (1937) and, yolk, albumin and shell percentage before stimulation of the data. Semen was collected from cocks and artificially inseminated to hens (cock/$^{\circ}$hens) two times per week. Also, monthly semen samples were individually collected by the massage method from all cocks. A small droplet from each cock semen was placed on a warm slide, covered with a cover slide and examined for sperm motility microscopically at 100x magnification (Melrose and Laing, 1970). Eosin-Nigrosine stain was used to determine the percent of morphologically dead spermatozoa (Lake and Stewart, 1978). Sperm-cell concentration was determined using the spectrophotometer density meter technique with diluted semen samples (1:250) as described by Lake and Stewart (1978).

At the end of experimental period blood samples were collected from 3 hens per treatment (5 ml/ hen) from the brachial vein and transferred into tube. No coagulated blood was used for hemoglobin determination. Serum samples were assigned for determination of total protein, albumin, glucose, cholesterol, high density lipoprotein cholesterol (HDL) and tri-glycerdes mg/dl using the suitable commercial chemical kits. Globulins were estimated by subtraction of albumin value from total protein value of each sample. At 34 weeks of age, nine birds from each treatment were immunized by intravenously injection with 1.0 ml of suspension of sheep red blood cells (SRBC'S) 7% in sterile saline (Yamamoto and Glick, 1982). Seven days following antigen challenge, blood samples were collected. Approximately 2.0 ml of blood was drawn from the brachial vein of each bird. It was allowed to clot to provide serum for antibody titer. Humoral immune response to SRBC'S was measured using micro haemagglutination technique.

At the end of the experimental period, 3 birds from each treatment were randomly chosen and individually housed in metabolic cages to determine the digestibility coefficient of nutrients of the experimental diets. The proximate analyses of feed and dried excreta were determined according to the official methods, **A.O.A.C. (1990)**. Fecal nitrogen was determined according to **Jakobson et al. (1960)**. Urinary organic matter was calculated according to **Abou – Raya and Galal (1971)**.

The economical efficiency (EE) of the experimental treatments was estimated depending on feeding cost and price of egg produced.

The data pooled through the experiment were proceed by General Linear Model procedures (GLM) described in SAS User's Guide (SAS, Institute, 2003). Mortality percentages were analyzed by using Chi - square test. Differences among treatment means were separated by Duncan's new multiple-range test (Duncan, 1955).

RESULTS AND DISCUSSION

Productive performance:

Results in Tables (4, 5 and 6) showed the effect of dietary supplementation of either betaine, vitamin C, folic acid or their mixtures during summer months on performance of Matrouh layers. Final body weight, egg production and egg mass were significantly (P<0.05) increased and feed conversion was improved with the diet supplemented with betaine compared with the control group from 24-36 weeks of age. Also, body weight at 32 or 36 weeks, feed conversion , egg production and egg mass were significantly (P<0.05) improved with the diet supplemented with betaine, vitamin C and folic acid together or in combinations compared to the control group during the most of experimental periods. However, feed intake and egg weight did not influence by experimental dietary supplementation or their mixtures during all the experimental periods. These results are in agreement with that Tollba *et al.* (2007) who found that significantly (P<0.05) increased in live body weight of brides (from 12 to 16 wks) fed diets supplemented with betaine during hot climate stress. The improving performance due to betaine supplementation could be attributed to several reasons, e.g. as methyl donor group, its diverse physiological properties that could improve gut environment and thus enhance the ability of the chicks to withstand coccidial infection, reduce intestinal membrane damage, dehydration, diarrhea and mal-digestion and\or absorption (Kettunen *et al.* 2001). In addition, Sayed and Downing (2011) found that the addition of betaine at an inclusion rate of 500 mg/L in drinking water improved body weight of broiler chicks under heat stress. Moreover, Ajakaiye *et al.* (2010) reported that increased feed intake and live weight gain in broilers and transported layer hens supplemented with vitamin C and/or E, during the hot summer season, respectively. The observed reduction in live weight particularly in control group could probably be due to the combinations of high ambient temperature and relative humidity which aggravated the intensity of cell damage in the birds. The increase in egg mass is due to an improvement in egg number. In this respect results reveal that no significant effect of treatments on egg weight, since the enhanced egg mass values are due to higher egg number, being more in females fed diets supplemented either betaine, vitamin C, folic acid or their mixtures. Furthermore, Pavlik *et al.* (2009) reported that reduced feed intake was the major cause of poor live weight, and reduced egg morphometric profiles, because enhanced enzymatic and non-enzymatic antioxidants systems were duly overwhelmed at both high ambient temperature and relative humidity. Ajakaiye *et al.* (2011) found that the White Leghorn layers fed diets supplemented with either150 mg vitamin C, vitamin E/kg diet or their mixtures recorded significantly ($P<0.05$) higher live body weight than those fed the control. These results are in agreement with those obtained by Kanazawa (2007) found that heat stress induces oxidative damage through producing free radicals such as O and OH that caused pro-inflammatory cytokinez, IL-1, IL-6, and TNFα. and producing free radical increases the expression of vascular endothelial growth factor (VEGF). Pro-inflammatory cytokinez and VEGF causes heart failure and blood circulation dysfunction leading to death (Vila *et al.* 2007). Moreover, El-Gendi *et al.* (1999) who reported that pullets fed diets supplemented with vitamin C had the highest feed consumption, egg mass and egg weight when compared with control. The beneficial effect of vitamin C supplementation may be attributed to activating thyroid gland which influenced by the feed intake (El-Fiky, 1998). As well as effects of vitamin C on feed conversion may be due to that vitamin C helps to decrease body temperature and plasma corticosterone concentration.

Folic acid in poultry diets led to a positive role on improving egg production and feed conversion efficiency (Liu and Feng 1992). Folic acid status is linked to increase serum levels of the sulfur amino acid homocystine due to the role of folic acid that play as co-factor in the remethylation of homocystine to form methionine (House *et al.*, 1999). In addition, folic acid plays an important role in amino acid and DNA metabolism and its deficiency causes severe defects in DNA replication and repair (McDowell, 1989). Sahin *et al.* (2003) noticed that supplementing a combination of vitamin C and folic acid may offer a practical protective in preventing heat stress related to depression in performance of broiler Japanese quail.

Egg quality:

Egg quality and egg components of Matrouh layers as affected by either dietary supplementation of betaine, vitamin C, folic acid or their mixtures during hot summer condition are presented in Table (7). Supplementation of betaine, vitamin C or folic acid together or in combinations were significantly increased ($P < 0.05$) the shell thickness, Haugh units and shell to egg weight compared to the control group. It is worth noting that, egg shape index, yolk (%) and albumin (%) were not significantly affected by dietary supplementation of betaine, vitamin C and folic acid alone, together or in combinations. These results agreed with those of Pavlik *et al.* (2009) who found that exposure of hens to high temperatures resulted in significant decrease in egg quality (shell weight, shell thickness, and specific gravity) when the birds were exposed to heat stress. These finding could be due to the reduction in feed consumption. The adverse effect of high environmental temperature on egg shell quality has been well documented (Mahmoud *et al.* 1996). The decrease in shell quality in the current study may be partially due to a reduction in plasma calcium. It has been reported that plasma calcium level was significantly decreased in laying hens (Mahmoud *et al.* 1996) when the birds were exposed to high temperatures. Also, Cheng *et al.* (1990) reported that shell weight per unit surface area showed a small increase with supplementary ascorbic acid (0, 100, and 200 ppm) and values (in Haugh units) were increased by ascorbic-acid at a level of 200 mg/kg with low relative humidity. Desoky (2008) found that dietary supplementation of vitamin C (200 mg vitamin C/kg diet) or vitamin E(150 mg vitamin E/kg diet) alone or combined showed high ability of alleviating the negative effect of heat stress and improved egg quality. Ajakaiye *et al.* (2011) found that egg yolk and egg albumen weights in groups administered with vitamins E and C+ E were significantly high as compared with the control group.

Semen physical characteristics:

Sperm motility, dead spermatozoa, and sperm cell concentration of Matrouh cockerels as affected by dietary supplementation of either betaine, vitamin C, folic acid or their mixtures during hot summer condition are presented in Table (8). Supplementation of betaine, vitamin C or folic acid together or their combinations were significantly ($P < 0.05$) increased sperm motility and decreased dead spermatozoa compared with the control group. While, supplementation of betaine, vitamin C and folic acid in combinations were significantly increased ($P < 0.05$) sperm cell concentration as compared with control group.

These results are in agreement with Hood (1999) who reported that heat exposure caused an increase in the percentage of dead sperm (29.1%) and a decrease in the sperm quality index (SQI) (10.2%). Whoever, Monsi and Onitchi (1991) supplemented the feed of heat-stressed broiler breeders with 0, 125, 250 or 500 ppm of ascorbic acid and who found that semen volume, total sperm, and motile sperm per ejaculate were significantly increased due to the addition of ascorbic acid. Noll (1997) reported that improved sperm cell concentrations in males and more eggs per hen when turkey breeder diets were supplemented with 200 mg per kg of vitamin C. This improved reproductive performance was noted in spite of environmental temperature fluctuations. In addition, Wallock *et al.* (2001) reported that folic acid might be vital to proper sperm development because it is required for the production of DNA.

Immune response to sheep red blood cells (SRBC'S):

Results of immune response to sheep red blood cells (SRBC'S) are presented in Table (8). Supplementation of either betaine, vitamin C, folic acid or their mixtures was significantly increased ($P < 0.01$) antibody titer against SRBC'S compared with those fed the control diet. These results agree with the findings of Zulkifi *et al.* (2000), who showed that heat stress caused a reduction in antibody synthesis. Naseem *et al.* (2005) reported that ascorbic acid improved immune response in birds under heat stress and disease condition. This reduction could be indirectly due to an increase in inflammatory cytokines under stress which stimulates the hypothalamic production of corticotrophin releasing factor (Sapolsky *et al.*, 1987). Corticotropin releasing factor is known to increase adrenocorticotropic hormone from the pituitary; adrenocorticotropic hormone then stimulates corticosterone production from the adrenal gland, which inhibits antibody production (Gross, 1992).

Blood hematological and biochemical parameters:

Blood hematological, biochemical parameters of Matrouh layers as affected by dietary supplementation of either betaine, vitamin C, folic acid or their mixtures during hot summer condition are presented in Table (9). Supplementation of betaine, vitamin C or folic acid together or in combinations significantly increased ($P < 0.05$) hemoglobin, total protein and globulin and significantly decreased ($P < 0.05$) serum glucose, cholesterol, high density lipoprotein cholesterol (HDL) and tri-glycerdes compared with the control group in layer. It is of interest to note that, supplementation of betaine, vitamin C and folic acid alone had no significant effect on serum albumin, glucose, cholesterol, HDL and tri-glycerdes concentration (Table 9).

Increasing hemoglobin, total protein and globulin may indicate that an enhancement of immunity occurred corresponding to feeding betaine, vitamin C and folic acid as a result of improving feed conversation, absorption and utilization of nutrients. The use of Vitamin

C has proved to reduce the serum cholesterol level and to alleviate the effects of heat stress in broiler quails (Gursu *et al.*, 2004). These results agree with the findings of Tollba *et al.* (2007) who reported that the improvement in serum total protein, albumin and globulin due to betaine and/or choline supplementation indicating further evidence about the role of methyl donor groups in protein metabolism. Similarly, Sahin *et al.* (2002) reported that serum glucose, cholesterol, and trygliceride decreased, whereas total protein, albumin concentrations increased when diet supplemented with vitamin C and folic acid. Moreover, Kutlu and Forbes (1993) reported that vitamin C supplementation to the diet increased plasma protein concentration whereas blood glucose and cholesterol concentrations, markedly decreased in heat-stressed (36°C) broilers. A likely mechanism by which vitamin C causes a reduction in corticosterone concentration is through inhibitory effect of vitamin C on glucocorticoid synthesis, and it has been postulated that the improved performance of poultry results from a decrease in protein-derived gluconeogenesis (McDowell, 1989). Increases in concentrations of glucose may be attributed to increased glucocorticoid secretion, which increases glucogenesis (McDowell, 1989). Dietary vitamin C may reverse these changes, presumably by reducing the secretion and/or synthesis of glucocorticoids. In addition, the magnitude of the results was greater when betaine, vitamin C and folic acid were supplemented together, indicating a possible additive effect of the three supplements. The similar effects of betaine, vitamin C and folic acid on most of the parameters could be due to the similarity between the role of betaine, vitamin C and folic acid as antistress agents. These compounds may act by different mechanisms or may have complementary roles as additive effects as we found.

Digestion coefficient:

The digestion coefficient of nutrients for the experimental diets is presented in Table (10). The digestion coefficient of crude protein (CP) and ether extract(EE) were significantly ($P<0.05$) increased with the diet supplemented with betaine alone or together with vitamin C or folic acid or in combinations as compared to the control group. El-Husseiny *et al.* (2007) reported that OM, CP, EE, CF and NFE digestion coefficients were significantly ($P<0.05$) increased with increasing betaine or folic acid levels in the diets of broiler chicks.

It is worth to note that betaine, vitamin C and folic acid supplementation had no significant effect on nitrogen-free extract (NFE), TDN and ME when compared with the control group. These results are in agreement with those of Abd-Elsamee *et al.* (2007) who found that Vit. C has been demanded as an antioxidant that scavenges the free radicals generated by heat stress in cell membranes and can protect the liver and other organs against oxidative damage. Therefore, supplemental Vit. C is necessary to maintain cellular metabolic function that greatly affects digestion and metabolism of nutrients.

Economic efficiency:

Data presented in Table (11) indicated that the best economic efficiency was obtained with layers fed diet containing of betaine plus vitamin C and follow in combinations of betaine, vitamin C and folic acid as compared to other groups and may be due to the better performance of this treatment. These results are generally in partial agreement with those reported by El-Husseiny et al. (2007) who mentioned that the highest economic efficiency was listed when diet contained the highest levels of betaine (0.5, 0.75 or 1.0 gm betaine/kg diet) and folic acid (0.5, 0.75 or 1.0 mg/ kg diet folic acid) in broiler chicks.

In conclusion:

from the nutritional and physiological points of view it could be concluded that the using of combinations of betaine, vitamin C and folic acid, in birds diets caused on improve in the productive, reproductive performance and economic efficiency under Egyptian hot summer condition. As well as the potential beneficial these effects could be due to their high ability of attenuating the oxidative damage induced by high temperature.

Table (1). Microclimatic data during the whole experimental period, under environmental condition.

Summer month	Averages temperature (^0C)		Averages RH (%)		Averages (THI)	
	Min*	Max**	Min*	Max**	Min*	Max**
June	22.5±0.37	35.0±0.40	25.7±1.11	75.5±1.66	20.63	33.44
July	23.7±0.21	34.1±0.29	34.7±1.08	83.1±1.16	21.82	33.07
August	24.5±0.21	35.1±0.22	34.9±1.14	83.6±0.93	22.46	34.05
Average	23.6±0.17	34.7±0.18	31.8±0.78	80.7±0.82	21.64	33.52

*=Minimum, **Maximum, RH=Relative humidity, THI=Temberature-humidity index

Table (2): Experimental design of the Matrouh layers strain fed basal diet and betaine, vitamin C or folic acid or their combination.

Groups	Control group	Treatment groups(kg/ diet)		
		Betaine	Vitamin C	Folic acid
1	Basal diet	-	-	-
2	Basal diet	1 gm	-	-
3	Basal diet	-	200mg	-
4	Basal diet	-	-	1mg
5	Basal diet	1 gm	200mg	-
6	Basal diet	1 gm	-	1mg
7	Basal diet	-	200mg	1mg
8	Basal diet	1 gm	200mg	1mg

Table (3): Composition and chemical analysis of the experimental diet fed during the laying period.

Ingredients	%
Yellow corn (8.5 %)	63.14
Soybean meal (44 %)	27.10
Limestone ($CaCo_3$)	7.55
Di-calcium phosphate	1.50
Dl-Methionine 99%	0.06
Salt (NaCl)	0.35
Vit + Min. premix*	0.30
Total	100.00
Chemical analysis:-	
a-Calculated analysis **:-	
ME, Kcal/Kg.	2722
Calcium, %	3.33
Available phosphorus, %.	0.40
Lysine, %.	0.88
Methionine,%	0.34
Methionine + cystine %.	0.64
Crude protein, %.	17.33
b-Determined analysis ***:-	
Crude protein,%.	16.76
Crude fiber,%.	3.98
Ash %.	6.38
EE	2.45

* Premix provides by kg: Vit A, 5500 IU; Vit E, 11 IU; Vit D3, 1100 IU; riboflavin, 4.4 mg; Ca pantothenate, 12 mg; nicotinic acid, 44 mg; choline chloride, 191 mg; vitamin B12, 12.1 ug; vitamin B6, 2.2mg; thiamine (as thiamine mononitrate), 2.2 mg; folic acid, 0.55 mg; d- biotin, 0.11 mg. Trace mineral (mg /kg diet): Mn, 60; Zn, 50; Fe, 30; Cu, 5; Se, 0.3

** Calculated according to NRC (1994).

*** Determined according to the methods of AOAC (1990).

Table (4): Effects betaine, vitamin C and folic acid on body weight and feed intake of the Matrouh layers from 24 to 36 weeks of age.

Items	Body weight (g), (weeks)				Feed intake(g/hen/day), (weeks)			
	24	28	32	36	24-28	28-32	32-36	24-36
Control(Basal diet)	1412.07±6.53	1463.73±17.94	1501.01±20.65^{d}	1524.86±16.56^{c}	98.30±0.93	98.13±0.86	106.90±0.72	101.11±0.29
Betaine	1408.77±6.87	1482.50±18.24	1545.59±19.16^{bcd}	1586.26±18.44^{ab}	98.27±0.74	100.17±0.78	103.67±0.69	100.70±0.15
Vitamin C	1412.17±8.57	1478.18±14.50	1541.94±15.18^{bcd}	1574.27±15.11^{bc}	97.83±0.65	99.43±1.31	103.77±1.11	100.34±0.22
Folic acid	1414.33±7.38	1477.97±15.14	1526.75±18.24^{cd}	1577.17±19.52^{abc}	97.37±1.03	99.50±0.91	103.83±0.84	100.23±0.40
Betaine + Vitamin C	1424.00±13.08	1509.17±16.00	1598.80±16.97^{ab}	1625.17±18.82^{ab}	97.40±0.70	99.37±1.04	104.23±1.13	100.33±0.28
Betaine+ Folic acid	1407.33±7.69	1501.53±17.42	1576.67±19.13^{abc}	1597.67±16.72^{ab}	97.70±0.87	101.70±1.08	105.87±1.26	101.76±1.03
VitaminC+Folic acid	1416.33±9.24	1489.17±11.95	1565.33±15.48^{abc}	1597.84±15.99^{ab}	97.30±1.10	101.33±0.95	106.47±1.17	101.70±0.76
Betaine +Vitamin C+ Folic acid	1418.00±12.22	1517.67±16.62	1604.80±16.63^{a}	1635.73±19.02^{a}	97.40±0.78	100.20±1.10	105.47±1.05	101.02±0.91
Sig.	NS	NS	*	*	NS	NS	NS	NS

Means having different letters at the same column are significantly ($P \leq 0.05$) different. * = $P<0.05$; ** = $P<0.01$; NS= Not significant.

Table (5): Effects betaine, vitamin C and folic acid on feed conversion and egg production of the Matrouh layers from 24 to 36 weeks of age.

Items	Feed conversion (g feed/g egg mass, (weeks)				Egg production (%), (weeks)			
	24-28	28-32	32-36	24-36	24-28	28-32	32-36	24-36
Control(Basal diet)	4.33±0.12^{a}	3.78±0.04^{a}	3.75±0.13^{a}	3.94±0.06^{a}	53.45±1.40^{c}	58.21±1.15^{c}	60.48±1.92^{b}	57.38±0.89^{d}
Betaine	3.97±0.14^{bc}	3.59±0.09^{abc}	3.38±0.09^{bc}	3.63±0.07^{bc}	57.86±1.80^{abc}	62.26±1.73^{abc}	65.48±1.87^{ab}	61.87±1.23^{abc}
Vitamin C	4.00±0.05^{abc}	3.67±0.12^{ab}	3.48±0.05^{abc}	3.71±0.13^{b}	57.14±1.15^{abc}	60.48±1.90^{bc}	63.33±1.75^{b}	60.32±1.11^{bcd}
Folic acid	4.12±0.07^{ab}	3.67±0.04^{ab}	3.54±0.13^{ab}	3.76±0.08^{ab}	55.36±1.56^{bc}	60.12±1.14^{bc}	62.38±1.95^{b}	59.29±0.91^{cd}
Betaine + Vitamin C	3.83±0.11^{bc}	3.34±0.08^{c}	3.20±0.08^{c}	3.44±0.03^{c}	59.40±1.75^{ab}	66.31±1.60^{a}	69.29±1.86^{a}	65.00±1.68^{a}
Betaine+ Folic acid	3.78±0.10^{bc}	3.46±0.06^{bc}	3.25±0.11^{bc}	3.48±0.04^{c}	60.12±1.17^{ab}	65.48±1.87^{ab}	69.17±1.33^{a}	64.92±0.75^{a}
VitaminC+Folic acid	3.89±0.11^{bc}	3.53±0.08^{abc}	3.42±0.10^{bc}	3.60±0.07^{bc}	58.45±1.17^{ab}	64.05±1.73^{ab}	66.07±1.56^{ab}	62.86±0.90^{ab}
Betaine +Vitamin C+ Folic acid	3.74±0.12^{c}	3.41±0.10^{bc}	3.23±0.07^{bc}	3.44±0.07^{c}	60.83±1.55^{a}	65.48±0.61^{ab}	69.29±1.69^{a}	65.20±1.00^{a}
Sig.	*	*	*	**	*	*	*	**

Means having different letters at the same column are significantly ($P \leq 0.05$) different. * = $P<0.05$; ** = $P<0.01$; NS= Not significant.

Table (6): Effects betaine, vitamin C and folic acid on egg weight and egg mass of the Matrouh layers from 24 to 36 weeks of age.

Items	Egg weight(g), (weeks)				Egg mass(g/hen/ day), (weeks)			
	24-28	28-32	32-36	24-36	24-28	28-32	32-36	24-36
Control(Basal diet)	42.50±0.08	44.56±0.13	47.24±0.20	44.76±0.10	22.72±0.57 ^{c}	25.94±0.46 ^{c}	28.57±1.01 ^{b}	25.69±0.46 ^{c}
Betaine	42.90±0.13	44.94±0.08	46.96±0.22	44.93±0.11	24.83±0.83 ^{ab}	27.98±0.73 ^{abc}	30.74±0.79 ^{ab}	27.80±0.56 ^{ab}
Vitamin C	42.83±0.10	44.83±0.10	47.09±0.24	44.92±0.12	24.47±0.47 ^{abc}	27.12±0.88 ^{bc}	29.82±0.76 ^{ab}	27.09±0.53 ^{bc}
Folic acid	42.76±0.15	45.09±0.13	47.07±0.23	44.98±0.09	23.67±0.66 ^{bc}	27.11±0.50 ^{bc}	29.37±1.03 ^{b}	26.66±0.40 ^{bc}
Betaine + Vitamin C	42.90±0.07	44.92±0.08	47.06±0.20	44.96±0.05	25.48±0.77 ^{ab}	29.78±0.67 ^{a}	32.61±0.94 ^{a}	29.22±0.77 ^{a}
Betaine+ Folic acid	43.04±0.10	44.92±0.07	47.21±0.25	45.06±0.11	25.87±0.48 ^{a}	29.41±0.83 ^{ab}	32.66±0.68 ^{a}	29.25±0.40 ^{a}
VitaminC+Folic acid	42.82±0.10	44.85±0.12	47.19±0.29	44.95±0.09	25.03±0.51 ^{ab}	28.72±0.70 ^{ab}	31.19±0.92 ^{ab}	28.26±0.45 ^{ab}
Betaine +Vitamin C+ Folic acid	42.93±0.06	44.91±0.10	47.19±0.24	45.01±0.08	26.11±0.67 ^{a}	29.40±0..69 ^{ab}	32.70±0.86 ^{a}	29.35±0.50 ^{a}
Sig.	NS	NS	NS	NS	*	*	*	**

Means having different letters at the same column are significantly ($P \leq 0.05$) different. * = $P<0.05$; ** = $P<0.01$; NS= Not significant.

Table (7): Effects of betaine, vitamin C and folic acid on egg quality and egg components of the Matrouh layers.

Items	Egg quality			Egg components (%)		
	Egg shape index%	Shell thickness (mm)	Haugh unit	Yolk	Albumin	Shell
Control	80.32±1.52	0.390±0.006 ^{b}	78.63±0.56 ^{b}	32.19±0.34	56.37±0.28	11.44±0.11 ^{b}
Betaine	76.49±2.95	0.412±0.007 ^{ab}	80.29±1.02 ^{ab}	32.08±0.51	56.06±0.63	11.86±0.13 ^{ab}
Vitamin C	79.74±2.29	0.408±0.008 ^{ab}	79.66±1.50 ^{ab}	32.05±0.71	56.08±0.62	11.87±0.16 ^{ab}
Folic acid	76.53±1.03	0.404±0.008 ^{ab}	80.80±0.92 ^{ab}	32.09±0.27	55.95±0.29	11.96±0.15 ^{ab}
Betaine + Vitamin C	75.17±3.47	0.428±0.009 ^{a}	82.26±1.09 ^{a}	31.85±0.56	55.64±0.74	12.51±0.29 ^{a}
Betaine+ Folic acid	76.62±2.45	0.416±0.007 ^{a}	81.69±0.40 ^{a}	31.91±0.30	55.84±0.48	12.25±0.36 ^{a}
Vitamin C +Folic acid	78.62±1.78	0.420±0.007 ^{a}	82.48±0.77 ^{a}	31.69±0.56	56.13±0.44	12.18±0.12 ^{a}
Betaine + Vitamin C+ Folic acid	75.90±1.87	0.428±0.006 ^{a}	82.50±0.63 ^{a}	30.78±0.58	57.06±0.55	12.16±0.16 ^{a}
Sig.	NS	*	*	NS	NS	*

Means having different letters at the same column are significantly ($P \leq 0.05$) different. * = $P<0.05$; ** = $P<0.01$; NS= Not significant.

Table (8): Effects of betaine, vitamin C and folic acid on semen quality of the Matrouh cockerels and immune response of the layers.

Items	Semen quality			Immune response to SRBC'S
	Sperm motility (%)	Dead spermatozoa (%)	Sperm cell concentration (X 109/ml)	
Control	75.00 ± 1.29^{b}	16.20 ± 1.05^{a}	3.74 ± 0.21^{b}	6.57 ± 0.29^{c}
Betaine	80.00 ± 1.29^{ab}	12.80 ± 0.83^{ab}	4.03 ± 0.24^{ab}	7.31 ± 0.21^{bc}
Vitamin C	79.00 ± 1.53^{ab}	12.40 ± 0.92^{b}	3.92 ± 0.17^{b}	7.29 ± 0.44^{bc}
Folic acid	78.00 ± 1.63^{ab}	13.00 ± 1.13^{ab}	3.85 ± 0.20^{b}	7.27 ± 0.15^{bc}
Betaine + Vitamin C	83.00 ± 1.00^{a}	11.60 ± 0.99^{b}	4.43 ± 0.13^{ab}	8.23 ± 0.18^{a}
Betaine+ Folic acid	82.00 ± 1.63^{a}	11.20 ± 0.87^{b}	4.36 ± 0.16^{ab}	7.73 ± 0.26^{ab}
Vitamin C +Folic acid	81.00 ± 1.53^{a}	10.40 ± 0.84^{b}	4.34 ± 0.10^{ab}	7.83 ± 0.20^{ab}
Betaine + Vitamin C+ Folic acid	83.00 ± 1.63^{a}	10.00 ± 0.93^{b}	4.68 ± 0.14^{a}	8.30 ± 0.20^{a}
Sig.	*	*	*	**

Means having different letters at the same column are significantly ($P\leq0.05$) different. * = $P<0.05$; ** = $P<0.01$; NS= Not significant.

Table (9): Effects of betaine, vitamin C and folic acid on blood hematological and biochemical parameters of the Matrouh layers.

Items	Hemoglobin (g/d)	Total protein (gm/dl)	Albumin (gm/dl)	Globulin (g/dl)	Glucose (gm/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	Tri-Glycerdes (mg/dl)
Control	10.43 ± 0.36^{c}	3.55 ± 0.23^{c}	1.57 ± 0.40	1.98 ± 0.19^{c}	122.29 ± 4.24^{a}	169.53 ± 8.35^{a}	124.88 ± 5.99^{a}	189.09 ± 8.92^{a}
Betaine	11.86 ± 0.31^{abc}	4.18 ± 0.18^{abc}	1.71 ± 0.31	2.47 ± 0.18^{abc}	110.63 ± 4.20^{ab}	160.55 ± 8.48^{ab}	106.76 ± 5.50^{ab}	170.61 ± 6.85^{ab}
Vitamin C	10.99 ± 0.39^{bc}	4.15 ± 0.23^{bc}	1.76 ± 0.32	2.39 ± 0.14^{abc}	112.46 ± 5.26^{ab}	166.02 ± 7.54^{a}	116.15 ± 5.75^{abc}	182.62 ± 10.89^{a}
Folic acid	11.12 ± 0.28^{bc}	4.13 ± 0.21^{bc}	1.79 ± 0.41	2.34 ± 0.21^{bc}	120.66 ± 5.04^{a}	162.89 ± 7.62^{ab}	113.88 ± 7.67^{abc}	181.33 ± 6.62^{a}
Betaine + Vitamin C	11.96 ± 0.29^{ab}	4.38 ± 0.23^{ab}	1.74 ± 0.43	2.64 ± 0.22^{ab}	101.66 ± 6.53^{b}	137.89 ± 8.73^{bc}	98.89 ± 7.42^{bc}	155.08 ± 8.23^{b}
Betaine+ Folic acid	12.17 ± 0.25^{a}	4.66 ± 0.22^{ab}	1.84 ± 0.19	2.82 ± 0.18^{ab}	100.49 ± 5.77^{b}	138.28 ± 7.13^{bc}	93.28 ± 6.37^{c}	166.54 ± 6.45^{ab}
Vitamin C +Folic acid	11.73 ± 0.31^{ab}	4.37 ± 0.20^{ab}	1.71 ± 0.31	2.66 ± 0.20^{ab}	100.09 ± 4.36^{b}	137.11 ± 8.31^{bc}	95.55 ± 4.97^{bc}	152.87 ± 5.94^{b}
Betaine + Vitamin C+ Folic acid	11.99 ± 0.21^{ab}	4.89 ± 0.25^{a}	1.91 ± 0.24	2.98 ± 0.17^{a}	99.91 ± 4.89^{b}	132.03 ± 7.30^{c}	94.15 ± 3.01^{b}	153.05 ± 6.54^{b}
Sig.	*	*	NS	*	*	*	*	*

Means having different letters at the same column are significantly ($P\leq0.05$) different. * = $P<0.05$; ** = $P<0.01$; NS= Not significant.

Table (10): Effects of betaine, vitamin C and folic acid on digestibility trials of the Matrouh layers .

Items	CP	EE	CF	NFE	TDN	ME
Control	79.20±0.17[d]	77.20±0.17[b]	23.97±0.20	79.23±0.22	64.84±0.14	2710.35±6.02
Betaine	79.95±0.21[ab]	78.33±0.19[a]	24.23±0.20	79.47±0.19	65.20±0.09	2725.20±3.65
Vitamin C	79.30±0.23[cd]	77.37±0.19[b]	23.98±0.19	79.30±0.23	64.94±0.17	2714.55±7.26
Folic acid	79.37±0.27[bcd]	77.43±0.17[b]	24.03±0.18	79.30±0.21	64.92±0.14	2713.78±5.74
Betaine + Vitamin C	79.43±0.15[bc]	78.37±0.22[a]	24.20±0.15	79.53±0.26	65.24±0.20	2727.12±8.52
Betaine+ Folic acid	79.88±0.15[abc]	78.07±0.19[a]	24.07±0.24	79.63±0.18	65.26±0.06	2727.77±2.64
Vitamin C +Folic acid	79.97±0.18[ab]	78.00±0.15[a]	24.00±0.23	79.40±0.23	65.02±0.19	2717.87±7.79
Betaine + Vitamin C+ Folic acid	80.10±0.17[a]	78.13±0.18[a]	24.03±0.20	79.43±0.27	65.16±0.15	2723.70±6.27
Sig.	*	*	NS	NS	NS	NS

Means having different letters at the same column are significantly ($P \leq 0.05$) different. * = $P<0.05$; ** = $P<0.01$; NS= Not significant

Table (11): Economic efficiency of the Matrouh layers as affected by betaine, vitamin C and folic acid at 36 weeks of age.

Items	Control	Betaine	Vitamin C	Folic acid	Betaine + Vitamin C	Betaine+ Folic acid	Vitamin C +Folic acid	Betaine + Vitamin C+ Folic acid
Egg number	48.20	51.97	50.67	49.80	54.60	54.53	52.80	54.77
Price/egg (LE)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total revenue (LE)[1]	24.10	25.99	25.34	24.90	27.30	27.27	26.40	27.39
Total feed intake/hen(kg)	8.49	8.46	8.43	8.42	8.43	8.55	8.54	8.49
Price/Kg feed (LE)	1.71	1.74	1.73	1.73	1.76	1.76	1.75	1.78
Total feed cost (LE)	14.52	14.72	14.58	14.57	14.84	15.05	14.95	15.11
Fixed hen (LE)[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Total cost hen (LE)	16.52	16.72	16.58	16.57	16.84	17.05	16.95	17.11
Net revenue/hen (LE)[3]	7.58	9.26	8.75	8.33	10.46	10.22	9.46	10.27
Economic efficiency (EEf)[4]	45.90	55.41	52.77	50.30	62.14	59.93	55.80	60.03

1- Total revenue = Egg number / hen X Price/egg (LE)
2- Fixed hen (LE) = Rearing cost .
3-Net revenue/hen (LE) = Total revenue - Total cost/hen.
4-EEf = Net revenue/hen (LE) / Total cost/hen (LE).

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الملخص العربي

تأثير إضافة البيتاين وفيتامين ج وحامض الفوليك للعليقه علي الأداء الإنتاجي والتناسلي لسلالة دجاج مطروح تحت ظروف الصيف الحار في مصر

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أجريت هذه الدراسة لدراسة تأثير إضافة البيتاين وفيتامين ج وحامض الفوليك منفردا أو معا أو مجتمعين علي الأداء الإنتاجي والتناسلي ومعاملات الهضم والكفاءة الاقتصادية لسلالة مطروح في الفترة من ٢٤-٣٦ اسبوع من العمر تحت ظروف الصيف الحار في مصر (شهر يونيو حتى شهر سبتمبر ٢٠٠٨).

وقد استخدام في هذه الدراسة ٢٤٠ دجاجة بياضة ، ٤٨ ديك من سلالة مطروح (سلالة محلية) قسمت عشوائيا إلي ثماني مجموعات بكل مجموعة ٣٠ دجاجات بياضه وستة ديوك، وكانت الدجاجات البياضة بكل مجموعة متساوية تقريبا في متوسط وزن الجسم. وكان الحد الأدنى والحد الأقصى لمتوسط درجة حرارة العنبر أثناء التجربة يتراوح بين ٢٣,٦،٣٤,٧ م° والرطوبة النسبية بين ٣١,٨،٨٠,٧%.

أظهرت النتائج المتحصل عليها تحسن معنوي($P<0.05$) لكل من وزن الجسم عند٣٢، ٣٦ أسبوع ومعدل التحويل الغذائي ، والنسبة المئوية لإنتاج البيض، كتلة البيض وذلك للطيور المغذاة علي البيتاين منفردا او مجتمعا مع فيتامين ج وحامض الفوليك بالمقارنة مع مجموعة الكنترول من ٢٤- ٣٦ أسبوع من العمر.

كما اظهرت التجربة أن إضافة البيتاين وفيتامين ج وحمض الفوليك معا أو مجتمعين زاد معنويا ($P<0.05$) كل من سمك القشرة ووحدات هاف ووزن القشرة لوزن البيض و% حيوية الحيوانات المنوية والهيموجلوبين والبروتين الكلي والألبيومين والاستجابة المناعية للطيور في سيرم الدم بينما الإضافة معا أدت إلي انخفاض معنوي ($P<0.05$) لكل من % للحيوانات الميتة الجلوكوز والكلسترول والكلسترول عالي الكثافة والجلسريدات الثلاثية مقارنة بمجموعة الكنترول تحت إجهاد حرارة الصيف. أظهرت النتائج ايضا زيادة معنوية في معامل هضم الألياف والدهن($P<0.05$) مع إضافة البيتاين منفردا أو مع فيتامين (ج) أو حامض الفوليك أو جميعا مقارنة بمجموعة الكنترول تحت إجهاد حرارة الصيف. كما إن أفضل كفاءة اقتصادية للدجاج البياض تم الحصول عليها مع اقل معدل للنافق لانتاج البيض في المجموعة المغذاة علي البيتاين وفيتامين (ج) وحامض الفوليك مجتمعا تحت إجهاد حرارة الصيف.

توضح نتائج هذه الدراسة أن تغذية طيور سلالة مطروح المحلية علي علائق محتوية علي البيتاين وفيتامين ج أو حمض الفوليك مجتمعا أدي إلي تحسين معظم الصفات الإنتاجية والتناسلية لهذه السلالة وذلك تحت ظروف الصيف الحارة في مصر.