

## Effect of supplementation of encapsulated organic acid and essential oil Gallant<sup>®</sup> on some physiological parameters of Japanese quails

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### Abstract

The current study was conducted to investigate the effects of dietary supplements of organic acid and essential oil Gallant<sup>®</sup> on the growth hormone, glutathione, performance of growth, some biochemical parameters and intestinal histomorphology in quails. Japanese quails one-day-old (n=120) were distributed randomly into four groups included 10 / 3 replicates for each group. The G1, was control group that was fed on a basal diet. Quails of G2, G3, and G4 were fed on a supplemented diet Gallant<sup>®</sup> 300, 600 and 900g/ton, respectively. Administration of Gallant<sup>®</sup> 600 g/ton and 900g/ton to quail led to significant decrease in triglyceride while supplementation with 600 g/ton caused decrease in cholesterol. On the other hand, 300 g/ton caused an increase in final body weight and total weight through the duration of the experiment, as well as a decrease in total feed consumption and the best feed conversion ratio in all supplementation with 300 g/ton caused a significant growth hormone elevation. All feed additives didn't affect the level of glutathione. The addition of Gallant<sup>®</sup> additive groups. Interestingly, the addition of different doses of Gallant<sup>®</sup> to the diet increased villus length and width, crypt depth, villus / crypt ratio, percentage of goblet cell, apparent surface area, and intestinal epithelium thickness compared to the control group. It was concluded that dietary supplementation with different doses of Gallant<sup>®</sup> improved growth hormone, growth performance and intestinal histomorphology in Japanese quails, and dietary supplementation with organic acid and essential oil as alternatives to the growth promoter of antibiotics.

**Keywords:** Gallant<sup>®</sup>, Growth hormone, Glutathione, Performance parameters, Japanese quail

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### تأثير الإضافات الغذائية للحمض العضوي والزيت الأساسي المغلف Gallant<sup>®</sup> على بعض المعايير الفسلجية للسمان

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### الخلاصة

أجريت الدراسة الحالية لمعرفة تأثير الإضافات الغذائية للحمض العضوي والزيت الأساسي المغلف Gallant<sup>®</sup> على مستوى هرمون النمو الكلوتاتيون وأداء النمو وبعض المعايير الكيميوحيوية والشكل النسيجي للأمعاء في طائر السمان. وزعت طيور السمان بعمر يوم واحد بشكل عشوائي الى أربع مجاميع متضمنة 10 طيور/ 3 مكرارات لكل مجموعة. المجموعة الاولى غُذيت على العليقة الأساسية واعتبرت مجموعة السيطرة. أما المجموعة الثانية والثالثة والرابعة فقد غُذيت على الإضافات الغذائية Gallant<sup>®</sup> وبجرعة 300، 600، 900 غم/طن عليقة على التوالي. أدى إعطاء Gallant<sup>®</sup> بجرعة 600 و 900 غم/طن للسمان الى انخفاض معنوي في مستوى الكليسيريدات الثلاثية بينما سبب إعطاء Gallant<sup>®</sup> بجرعة 600 غم/طن الى انخفاض معنوي في مستوى الكوليسترول ومن ناحية أخرى أدى إعطاء 300 غم/طن ارتفاع معنوي في مستوى هرمون النمو مقارنة مع مجموعة السيطرة. لم تؤثر جميع الإضافات الغذائية في مستوى الكلوتاتايون. أدى إعطاء Gallant<sup>®</sup> بجرعة 300 غم/طن الى حدوث ارتفاع معنوي في وزن الجسم النهائي وزيادة الوزنية الكلية خلال

التجربة، بينما انخفض استهلاك العلف الكلي مع تحسن في معامل التحويل الغذائي في جميع المجاميع المعاملة مقارنة مع قيم السيطرة. أشارت البيانات الى أن الإضافات الغذائية في Gallant<sup>®</sup> وبجرع مختلفة أدت الى زيادة معنوية في طول الزغابات وعرضها وعمق الخبايا ونسبة الزغابات الى الخبايا والنسبة المئوية للخلايا الكأسية والمساحة السطحية الظاهرية وسمك ظهارة الأمعاء مقارنة مع مجموعة السيطرة. أستنتج من هذه الدراسة بأن الإضافات الغذائية وبجرع مختلفة من Gallant<sup>®</sup> قد حسنت من مستوى هرمون النمو والشكل النسجي للأمعاء ومعايير أداء النمو للسمن الياباني وتعد الإضافات الغذائية للحمض العضوي والزيت الأساس كبداية للمضادات الحياتية المحفزة للنمو.

## Introduction

Japanese quails have economic importance as a source of meat and egg production; however, quail's production has not been infinitely employed or considered among the poultry industry (1). Breeding the quail is now a widespread manner and could help to overcome the current gap in meat supply and demand (2). The strange taste and characteristics of quail meat have led to increased consumer interest (1). The enhancement of growth performance and feed conversion ratio could be reached in the poultry industry by usage of specific feed supplements (3). The using of feed additive has been an essential part of getting success in poultry production. Familiar feed supplementation used in poultry diet include antimicrobials antioxidants, emulsifier, binders, pH control agents and coenzyme (4). There are widespread uses of antibiotic alternatives like organic acids, probiotics, medicinal plants, and organic acid; despite the higher price of these additives, these products have more supporters among costumers (5). Generally, the advantage of essential oils as a feed supplementation has mostly intensive on the influence of animal performance (6). Essential oils are specific for their strong smell and varied composition, chemically, essential oils are complex and very variable mixtures of components that belong to two groups: terpenoids (monoterpenes and sesquiterpenes), aromatic compounds (aldehyde, alcohol, phenol, and methoxy derivative and so on) and terpenoids (isoprenoids) (7). The results of Ahmad *et al.* (8) indicated that the phytogenic feed additive administration as thyme and star anise oils to the broiler chicken diets produce a linear rise in the apparent ileac digestibility of calcium and phosphorus, respectively. In addition, thus they concluded that the improvement in the digestibility of nutrients could be caused by the stimulatory effect of the phytogenic feed, which improved the surface area of absorption in the intestine and endogenous digestive enzymes in addition to antioxidants and lipid metabolism activities (9). Another potential feed additive are organic acids which showed a positive result on poultry production and improving intestinal health; thus, the bird can maximize nutrient absorption. The organic acids also stimulate the pancreatic secretion and provide better intestinal villus integrity (10).

The current study aimed to investigate the effect of dietary supplementation Gallant<sup>®</sup> a blend of Organic Acid

(OA) and Essential Oil (EO) supplementation, on growth performance, intestinal histomorphology, some biochemical parameters, and growth hormone in Japanese quails.

## Material and methods

### Birds

The study was carried out at the animal house located at the College of Veterinary Medicine, University of Mosul, Mosul, Iraq. A total of 120 one-day-old Japanese quails, were ordered from the Agriculture College, University of Mosul. Quails were divided randomly into four groups 10 birds each, where four treatments of three replications were applied. The quails were housed in floor pens 1x2 meter and the temperature of the room fluctuated from 22 °C to 28 °C. The relative humidity was between 48% - 58% and provided by constant light 17 hours/day and clean drinking water *ad libitum* throughout the rearing periods of 7 weeks. The constituent and nutrient taken under the levels of the basal diet recommendation NRC (11). All birds were kept under standard hygienic conditions.

### Gallant<sup>®</sup>

Consist of protected or encapsulated organic acids of formic acid, acetic acid and butyric acid, while essential oils thymol, carvacrol,  $\beta$ -cymene, borneol and myrcene coated with a matrix of triglyceride and produced by [www.jefo.com](http://www.jefo.com). The recommended dose is 300 gm/ton of feed. The feed additive used in the research were homogenate with the feed prior to its providing.

### Experimental design

Quails were divided into 4 groups: G1, the control group, fed on a basal diet. Quails of G2 were fed on a basal diet supplemented with Gallant<sup>®</sup> 300 g/ton, G3 was fed on a basal diet supplemented with Gallant<sup>®</sup> 600 g/ton, while quails in the G4 were fed on a basal diet supplemented with Gallant<sup>®</sup> 900 g/ton.

### Internal organs weight

At the end of the experiment, three quails / replicate were randomly chosen and slaughtered. The weights of the liver and heart were recorded. The weight of the organ/100 g body weight.

### **Growth performance parameter**

The initial body weight (g), final body weight (g), total feed consumption /quail (g) and feed conversion ratio (g/g) FCR were recorded on a weekly. First egg production and the average of eggs weight (g) were recorded on a daily basis.

### **Blood sampling**

During slaughtering, blood samples were collected and sera was separated at 3000 rpm by blood centrifugation for 15 minutes. Sera was stored at -20 °C until processing time. The parameters analyzed included: serum: cholesterol, triglyceride and total protein using commercial kits (Biolabo / France) and growth hormone measured using GH Chicken ELISA kit (Al-shkairate Medical Supply Establishment /Jordan). Ellman's procedure (12) was followed to determine the level of glutathione.

### **Intestinal histomorphological examination**

The intestinal morphometric variables including villus length and width, crypt depth, villus/crypt ratio, appearance surface area, goblet cell percentage and intestinal wall thickness. A small intestine segment of 3 cm was separated and fixed in 10% formalin buffered. The small intestine segment was embedded in paraffin. A 5 µm section of each sample was put on the glass slide and stained with hematoxylin and eosin (13,14). All parameters were measured using the USB 2.0 color digital image camera (Scope Image 9.0-China) provided with image processing software. The camera software was calibrated to all Microscope-Olympus-CX31 lenses using 0.01 mm stage micrometer (ESM-11/Japan). The peak of each villus was calculated from the highest of the villus to the crypt transition and the crypt deepness was described as the invagination between two villi while the width of villi was estimated by measuring the average of the base and tip of the villus, the peaks of 40 villi and the depths of 40 crypts were calculated for each bird. All the above parameters arranged in tables to achieve comparison among different groups.

### **Statistical analysis**

Data were analyzed by means of one - way analysis of variance (ANOVA). The significance of differences between means was tested via the Duncan multiple range test at  $P < 0.05$  (15).

### **Results**

The effect of the dietary additive of Gallant<sup>®</sup> on biochemical parameters in Japanese quails is summarized in Table 1. A significant decrease in cholesterol level was observed in Gallant<sup>®</sup> 600 g/ton compared with the control group. On the other hand, administration of Gallant<sup>®</sup> 300 gm/ton and Gallant<sup>®</sup> 900 gm/ton for 7 weeks did not affect

cholesterol level significantly and return to control value. Furthermore, there was no significant variation in cholesterol level between Gallant<sup>®</sup> 300 g/ton and 900 g/ton by compared with the control group, supplementation of Gallant<sup>®</sup> 300 g/ton didn't affect triglyceride level, but supplementation with Gallant<sup>®</sup> 600 g/ton and 900 g/ton caused a significant decrease in triglyceride level. In addition, there is no obvious change in triglyceride values between Gallant<sup>®</sup> 600 g/ton and 900 g/ton. The administration of Gallant<sup>®</sup> 300 g/ton and 600 g/ton additives did not noticeably influence the total serum protein level, but Gallant<sup>®</sup> 900 g/ton resulted in a significant rise in the total protein related to the control group. No important change in total serum protein levels between Gallant<sup>®</sup> 300 g/ton and 600 g/ton.

Table 2 determination of liver and heart weight at the end of the experiment and represented in Table 2. All feed additive didn't affect significantly on visceral organs weight. The results indicated that dietary supplementation of Gallant<sup>®</sup> 300 g/ton resulted rise in growth hormone level significantly compared to control value. On the other hand, there was no significant variation between other supplemented groups on growth hormone level compared to the control group, nor was there any important effect between Gallant<sup>®</sup> 600 g/ton and Gallant<sup>®</sup> 900 g/ton. Glutathione level did not affect significantly in all supplemented groups similarity to control value.

Table 3 showed that all groups supplemented with Gallant<sup>®</sup> did not cause a significant difference in the initial weight compared with the control group; while the administration of Gallant<sup>®</sup> 300 g/ton increased significantly in final body weight and total body weight gain. In addition, other additive groups did not affect significantly the final body weight and total body weight gain compared to control one. The results revealed that total feed intake decline significantly in all additive groups accompanied with improved feed conversion ratio and the minimal best value was 900 g/ton as compared with the control group.

The intestinal histomorphology data indicated that administration of Gallant<sup>®</sup> 300 g/ton, 600 g/ton and 900g /ton in for 7 weeks caused a significant increase in both the villus length and width and similarity with the normal control value (Figures 1 and 2). Administration of Gallant<sup>®</sup> 300 g/ton caused a significant increase in crypt depth compared with other additive groups, while villus to crypt ratio and the percentage of goblet cell recorded the highest significant value at 600 g/ton compared with other groups. The data revealed a significant elevation of the intestinal epithelium thickness at the dose of 300 g/ton compared with the supplemented groups. On the other hand, the appearance surface area was significantly increased at Gallant<sup>®</sup> 600 g/ton as compared with the control group (Table 4).

Table 1: Effect of Gallant<sup>+</sup> supplementation on biochemical parameters in quails

Treatment	Mean ± SE		
	Cholesterol mg /dl	Triglyceride mg /dl	Total proteins g/dl
Control	263.7±8.06ab	196.1±3.98a	3.06±0.09b
Gallant <sup>+</sup> 300 g/ton	279.23±11.7a	207.4±8.03a	3.33±0.08ab
Gallant <sup>+</sup> 600 g/ton	245.7±11.6c	155.6±7.04b	3.16±0.08b
Gallant <sup>+</sup> 900 g/ton	274.4±5.4ab	146.6±4.23b	3.53±0.12a

Values using different letters in the column are significantly different P<0.05.

Table 2: Effect of Gallant<sup>+</sup> supplementation on organs weight, growth hormone and glutathione levels in quails

Treatment	Mean ± SE			
	Liver weight g/100 g.bw	Heart weight g/100 g.bw	Growth hormone ng/ml	Glutathione µmol/L
Control	2.29 ± 0.19a	0.86 ± 0.4a	0.394 ± 0.07b	0.157 ± 0.028 a
Gallant <sup>+</sup> 300 g/ton	2.59 ± 0.18a	0.91 ± 0.1a	0.516 ± 0.07a	0.199 ± 0.014 a
Gallant <sup>+</sup> 600 g/ton	2.12 ± 0.10a	0.92 ± 0.3a	0.470 ± 0.08ab	0.20 ± 0.005 a
Gallant <sup>+</sup> 900 g/ton	2.61 ± 0.32a	1.07 ± 0.1a	0.410 ± 0.06b	0.174 ± 0.012 a

Values using different letters in the column are significantly different P<0.05.

Table 3: Effect of Gallant<sup>+</sup> supplementation on growth performance parameters in quails

Treatment	Mean (g) ± SE				
	Initial weight	Final weight	Total weight gain	Total feed intake	Feed conversion ratio
Control	14.37±0.80a	216.33±3.17b	201.96±3.13b	623.73±1.84a	3.08±0.4a
Gallant <sup>+</sup> 300 g/ton	14.59±0.90a	225.63±1.29a	211.04±1.31a	586.63±1.33b	2.77±0.2b
Gallant <sup>+</sup> 600 g/ton	14.70±0.70a	219.53±0.4ab	204.82±0.48ab	518.16±1.24c	2.52±0.006c
Gallant <sup>+</sup> 900 g/ton	14.62±0.10a	218.56±2.31b	203.94±2.37b	408.3±1.40d	1.99±0.01d

Values using different letters in the column are significantly different P<0.05.

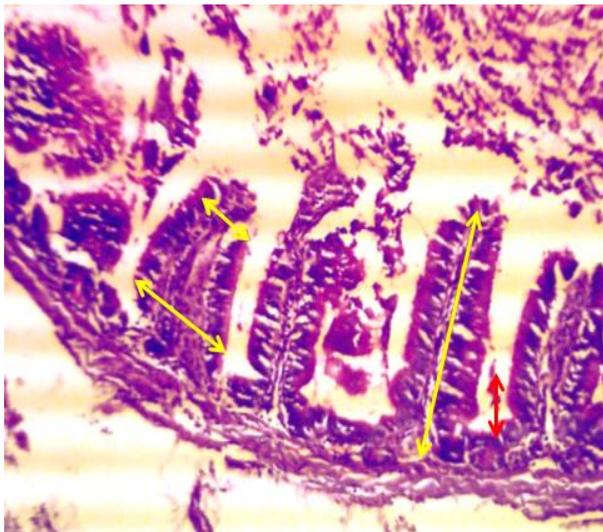


Figure 1: Villi photomicrographs in the ileum of quail fed with basal diet control, yellow arrow indicates length and width, red arrow indicates the depth of crypt, H&E, 10x.

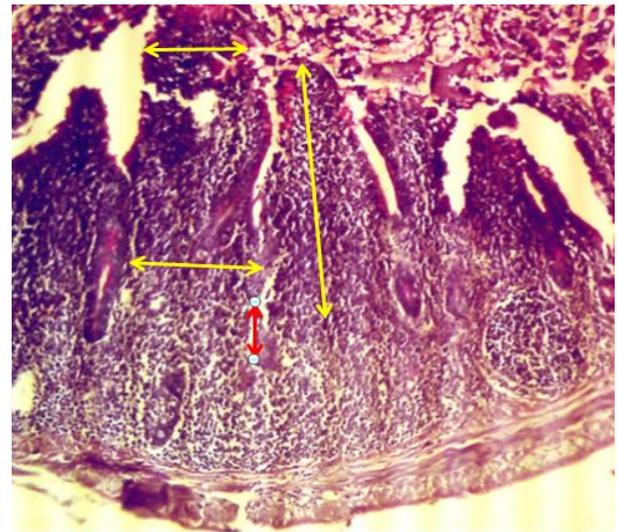


Figure 2: villi photomicrographs in the ileum of quail fed with basal diet and Gallant<sup>+</sup> 300 g/ton, yellow arrow indicates length and width, red arrow indicates the depth of crypt, H&E, 10x.

Table 4: Effect of Gallant<sup>®</sup> supplementation on intestinal histomorphology in quails

Treatment	Mean (µm) ± SE						
	Villus length	Villus width	Crypt depth	Villus / crypt ratio	% Goblet cell	Epithelium thickness	Appearance surface area
Control	156.6±6.2c	24.1±0.5d	40.7±1.4b	3.8±0.1c	48.5±0.6b	473.8±5.3b	3792±215d
Gallant <sup>®</sup> 300 g/ton	339.7±10.2a	39.6±0.4a	162.0±5.2a	2.1±0.1d	36.61±1.6c	580.6±19.2a	43454±385a
Gallant <sup>®</sup> 600 g/ton	230.4±5.8b	32.17±0.5c	35.6±0.9b	6.45±0.3a	54.1±0.7a	341.2±10.3c	7366±309c
Gallant <sup>®</sup> 900 g/ton	233.7±5.6b	35.1±0.6b	42.1±0.8b	5.7±0.2b	30.7±0.6d	490.9±24.6b	8464±234b

Values using different letters in the column are significantly different P<0.05.

Figure 3 demonstrating the first day of egg production was started at 42, 43, 44, 45 day of age, respectively, accompanied with average egg weights 6.5, 6.7, 6.8, 8.3 in Gallant<sup>®</sup> 300g/ton group compared with control group,

Gallant<sup>®</sup> 600g/ton and Gallant<sup>®</sup> 900g/ton where the first egg production started at 47 of age, and all groups continuous in eggs production to the end of experiment.

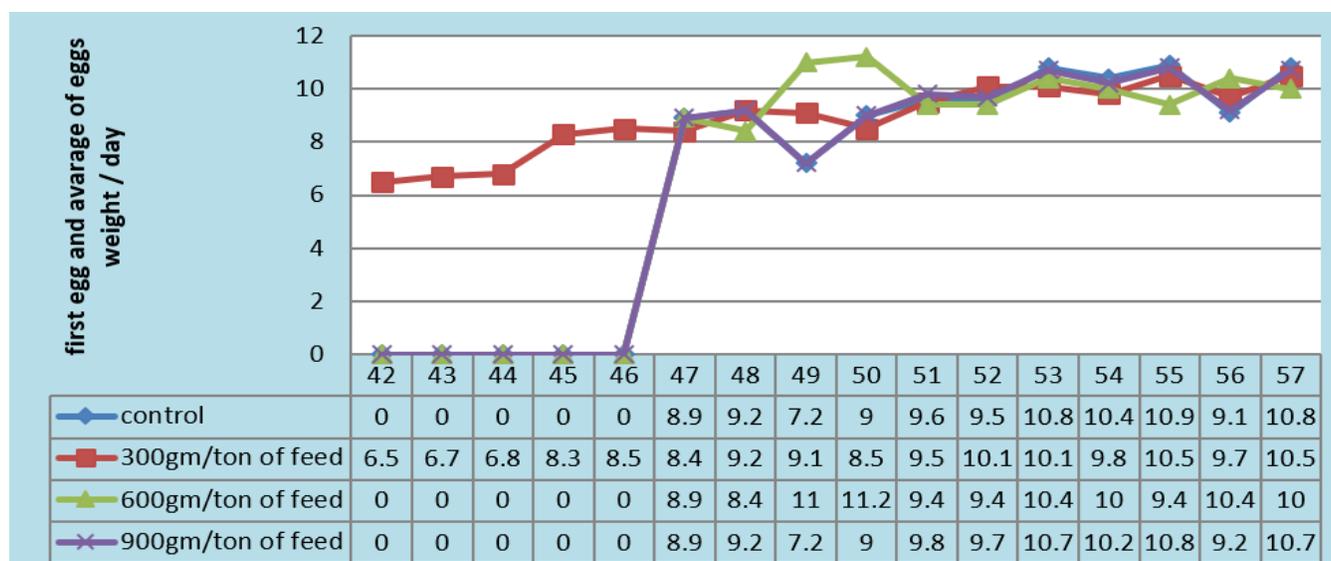


Figure 3: First egg production and the average of eggs weight/day.

**Discussion**

Gallant<sup>®</sup> consist of organic acids (formic acid, acetic acid, and butyric acid) and essential oils (thymol, carvacrol, B-cymene, borneol, and myrcene), thus, at least one of these components might be impacted on the changes of the studied parameters.

According to the blood analysis, supplemented Gallant<sup>®</sup> 600 g/ton with feed caused a significant decrease in cholesterol level, furthermore Gallant<sup>®</sup> at 600 g/ton and 900 g/ton with diet caused a significant decrease in triglyceride level. This result is consistent with a previous study recorded by Al-Mashhadani *et al.* (16), who found that birds feed on a diet having thyme essential oils and anise cause decrease in cholesterol concentration. Moreover, our result agreed with another work where a

blend of essential oils and organic acids supplementation at 0.25, 0.5 or 0.75 g/kg diet significantly reduced triglycerides and total cholesterol (17). The etiology of the decrease total cholesterol and triglycerides concentrations could be attributed to the reducing effect of essential oils on hepatic 3-hydroxy-3methylglutaryl coenzyme A (HMG-CoA) which is necessary for cholesterol creation in the liver. The mechanism of essential oils inhibits HMG-CoA reductive activity (18), which is an important regulatory enzyme in cholesterol synthesis and as a result the hypocholesterolemia influence (19).

Unlike other feed additives, the consumption of Gallant<sup>®</sup> 900 g/ton caused a significant increase in total serum protein. This finding is in harmony with (20) where adding thyme to Japanese quail's diets enhanced serum protein level in addition to globulin then albumin at 42 days

of age. The increase of globulin could be due to the immune stimulatory impact of the essential oil supplementation. In addition, another work indicated that there were undetectable changes in both the blood serum total protein and albumin concentrations after the adding of a mixture of essential oils and organic acids supplementation at 0.25, 0.5 or 0.75 g/kg, while blood serum globulin concentration was significantly increased (17).

Our results demonstrated that Gallant<sup>+</sup>® 300 g/ton dietary supplementation resulted in a significant elevation in growth hormone level. This result agreed with a previous work where a significant increase in the growth hormone was specified (21). The similarity to the findings of our study Hossein *et al.* (22) showed that ingestion of both Fermacto<sup>®</sup> and Protexin<sup>®</sup> in quail feed could stimulate growth hormone secretion. Moreover, our result did not show a significant change in glutathione level between groups; this result disagreed with Farag *et al.* (23) who found the relationship among antioxidants property of essential oils and its chemical composition. In addition, essential oils acting as active free radical scavengers and boosts the antioxidant system like vitamin E, glutathione peroxidase and superoxide dismutase (24). During the spring time, glutathione serum level was significantly increased associated with summer time at a value level P less than 0.05 (25). In the present study, the variation in administration duration of Gallant<sup>+</sup>®, its provided dose, and animal species might cause undetectable changes in glutathione level.

Administration of Gallant<sup>+</sup>® 300 g/ton caused a noticeable improvement in the final weight and total weight gain and decreased in total feed consumption accompanied by improvement in feed conversion ratio. Similar observations of improved growth performance were recorded in Japanese quail chicks where the diet was supplemented with a blend of essential oils and organic acids (16). Moreover, Soltan (26) revealed that organic acid mixture (formic acid and salt of butyric acid, propionic acid and lactic acid) supplementation at the level 780 ppm of laying hens diet improve live body weights. Furthermore, supplementation of organic acid enhanced growth performance when given in drinking water route (27). It has suggested that the enhanced growth performance is associated with the improved cell proliferation of the intestine and stimulating protein synthesis (28). In addition, organic acids especially formic, citric acids, acids propionic acids have properties to rise proteins besides amino acids digestibility and gastric proteolysis (29). The enhanced performance in the studied parameter is probably due to the beneficial effect formic acid and essential oil on the intestinal flora, this is confirmed by the current study by improvement in histomorphologic parameters because the villus

plays an important role in absorption processes of the small intestine (30).

The results reported here indicated that the addition of different doses of Gallant<sup>+</sup>® to the diet of Japanese quails increased the villus length and width, crypt depth, villus /crypt ratio, percentage of goblet cell, intestinal wall thickness and appearance intestinal surface area. Our results are in agreement with those obtained by Khaksar *et al.* (31), who demonstrated the effect of essential oil and thyme additive on intestinal histomorphology of broiler, others reported that birds fed a blend of essential oil from oregano anise and citrus peel had longer ileac villus height and more goblet cell (32). The rise number of goblet cell per villus might be attributed to villi grow, and goblet cell secretes mucous all over the gastrointestinal region that forms an adherent gel on the mucosal surface and may production an important role in epithelial cell repair (33), leading to the improved intestinal digestive function. Together crypt depth and villus height are important markers of chicken digestive system health and connected to the absorptive capacity of the intestinal mucous membrane (34). This changing in histomorphology of the intestine of the current study may enhance the action of two pancreas enzymes including  $\alpha$ -amylase and lipase in the intestinal tissue when using carvacrol in the diet of the older chicken these pointed out by Jamroz *et al.* (35). Similarly, showed that thymol and carvacrol might elevated intestinal lipase beside trypsin activities in broilers chicken (36).

It was observed that organic acid supplementation markedly increased the intestinal absorption area by promoting villus growth in height and in width at 42 days. In parallel, the goblet cells count was also significantly higher in the treated group whatever the intestinal segment. Moreover, the induction of these histological modifications appeared early (before the 21<sup>st</sup> day), transient in duodenum and jejunum but prolonged (after the 21<sup>st</sup> day) in the ileum part since the magnification rates calculated for ileum villi in supplemented birds for the 21-42 days period were significantly increase as compared with control. These results demonstrate a positive effect of organic acids on the intestinal mucosa (37).

The present study demonstrated that first egg production was started earlier in Gallant<sup>+</sup>® 300g/ton group compared with the control group. This result did not comply with results obtained by Świątkiewicz *et al.* (38) who reported that organic acid additive had no effect on the eggs production and average eggs weight compared with the control group. A previous study demonstrated that compounds like thymol, B-cymene, and carvacrol have strong antioxidant properties (39), hence Gallant<sup>+</sup>® acts as an antioxidant, thus reducing the quail stress. Therefore, Gallant<sup>+</sup>® supplementation may be useful in reducing corticosterone concentrations because it acts as an antioxidant, so Gallant<sup>+</sup>® act as an antioxidant, thus reduced

stress to the quails, therefore, Gallant<sup>®</sup> supplementation might be helpful in decreasing corticosterone levels because it acts as an antioxidant. Dragomir *et al.* (40) suggested that increased production of Hen-day eggs and egg weights during heat stress circumstances were attributed to aspirin suppressing prostaglandin synthesis, enabling full calcification of soft-shelled eggs. It also seems to be an inverse relationship between serum levels of corticosterone and LH as it suppresses the release of LH from pituitary cells (41). Therefore, it could be assumed that blocking prostaglandin synthesis through aspirin supplementation may be useful in decreasing corticosterone levels and thus increasing concentrations of LH and FSH, thereby activating ovaries to produce more eggs (42).

## Conclusion

Thus, we concluded from our study that dietary supplementation of organic acid and essential oil with different doses improved growth hormone, growth performance and intestinal histomorphology as well as hypocholesterolemia effects and could be used as growth promoters. It is believing that essential oil will play a enormous role in poultry industry development.

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