EFFECT OF USING BUTYRIC ACID ON PERFORMANCE OF JAPANESE QUAIL FED OPTIMAL AND SUB-OPTIMAL ENERGY AND PROTEIN LEVELS

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ABSTRACT

An experiment was conducted to study the effectiveness of dietary butyric acid glycerides supplementation as a growth promoter on Japanese quail (Coturnix coturnix japonica) performance, carcass characteristics, intestinal pH, bacteria enumeration and blood parameters. A total number of 360 unsexed day-old Japanese quail chicks were equally divided into four groups of three replicates each. Two starter-grower corn-soybean meal (C-SBM) basal diets were formulated to contain 24% CP and 2900 kcal ME/kg diet and 22% CP and 2750 kcal ME/kg diet, respectively. Also, two layer C-SBM basal diets were formulated to contain 20 % CP and 2900 kcal ME/kg diet and 18% CP and 2750 kcal ME/kg diet, respectively. Each of the four basal diets was either unsupplemented or supplemented with 2 kg/ton dietary butyric acid glycerides (BA). Therefore, four experimental treatments were used in both starting-growing and laying periods. At 42 days of age, a slaughter test was performed to determine carcass traits, edible giblets, lymphoid organs and intestinal microflora count and pH. Blood samples were taken and assayed to determine some serum blood parameters. At laying period, egg number, weight, mass and production as well as feed intake and conversion were recorded. At the end of the 90-day period, egg samples were taken and broken out to determine egg quality. During growing period, the results showed that supplementing BA to diet (24% CP and 2900 kcal ME/kg) significantly decreased feed intake, abdominal fat, counts of E coli and salmonella spp. in both ileum and caecum. However, it significantly increased pH and counts of Lactobacillus spp. in ileum and caecum as well as serum Ca and P concentrations. Supplementing BA to diet (22% CP and 2750 kcal ME/kg) significantly increased growth performance, thymus gland, Lactobacillus spp and pH in ileum and caecum. During laying period, the results showed that supplementing BA to diet (20 % CP and 2900 kcal ME/kg) significantly decreased feed intake, improved feed conversion ratio and increased egg albumen. However, Supplementing BA to diet (18% CP and 2750 kcal ME/kg) led to significant improvement in egg production traits, egg shape index, Haugh units, shell thickness, egg yolk and egg shell. From nutritional point of view, it was observed that using dietary butyric acid at a level of 2 kg/ton dietary in Japanese quail diets containing sub-optimal energy and protein levels helped in reducing microflora count, particularly pathogens and in turn, improving quail performance and immunity.

Key words: *Butyric acid, butyrate, performance, intestinal pH, carcass, blood serum, egg production, egg quality, quail.*

INTRODUCTION

Under modern system of poultry production, birds are inevitably exposed to considerable stress during their lifetime. The time immediately after hatching is also a period of stress. The gastrointestinal tract of newly hatched chicks is immature and sterile. It begins to develop their function and microflora when it starts to ingest feed. At this time, the chick is very susceptible to pathogenic microorganisms **(Adams, 2004)**. Under such circumstances, anti-microbial feed additives such as antibiotics are often used to suppress or eliminate harmful organisms in the intestine and to improve growth and feed efficiency **(Jin *et al.*, 1997)**.

Research has grown over the past years and aimed at alternative means to manipulate the gastrointestinal microflora in production livestock. Motivation for testing these alternatives comes from the fact that antibiotics inclusion in poultry diets as routine feed additives has been banned by the European Union because of the public concern over possible antibiotic residual effects and the development of drug resistant to disease causing bacteria **(Gunal *et al.*, 2006; Leeson, 2007; Yang *et al.*, 2009)**.

This has led to the application of non-antibiotics chemical substances **(Yang et al., 2009)**. A modern approach is needed to be based on dietary and nutritional, rather than pharmacological and conditioning agents. Therefore, there is a need to look for viable alternatives that could enhance the natural defense mechanisms of animals and reduce the massive use of antibiotics **(Verstegen and Williams, 2002)**. A way is to use specific feed additives such as organic acids to favorably affect animal performance through the modulation of the gut microbiota, which plays a critical role in maintaining host health **(Tuohy *et al.*, 2005)**.

Amongst the organic acids, short chain fatty acids (SCFAs) such as acetate, propionate and butyrate. Recent research has shown the positive effects of SCFAs on *Salmonella enteritidis* control **(Van Immerseel *et al.*, 2002; Van Immerseel *et al.*, 2005)** and their growth promoting effect on the beneficial intestinal microflora **(Dibner and Buttin, 2002; Van Immerseel *et al.*, 2004b; Hansen *et al.*, 2007)**. Bacterial cells take up undissociated fatty acids, and once these dissociate, the change in intracellular pH is usually bactericidal **(Van der Wielen *et al.*, 2002)**.

Butyric acid (BA) is one of such SCFAs, which considered the prime enterocytes energy source **(Antongiovanni *et al.*, 2007)** and necessary for the correct development of the gut associated lymphoid tissue **(Friedman and Bar-Shira, 2005)**. It has also higher bactericidal activity when it is undissociated **(Leeson, 2007)**. Bacterial cell take up undissociated fatty acids and once these acids dissociate, there is change in the intracellular pH leading to death of bacterial cells. However, the levels of SCFAs are quite low in the intestine and caeca of young chicks **(Van der Wielen, 2000)** and so the young animal may be the best candidates for dietary supplementation. In addition, positive beneficial effects of BA and butyric acid glycerides on production performance and carcass traits of broilers have been reported by **Leeson *et al.* (2005)** and **Antongiovanni *et al.* (2007)**.

However, there is little information available on butyrate in poultry. Hence, further studies are needed on the effects of butyrate supplementation in poultry. The present study was therefore; conducted to study the effect of butyric acid glycerides as a growth promoter on Japanese quail (*Coturnix coturnix japonica*) performance.

MATERIALS AND METHODS

Experimental birds and housing

Three hundred and sixty unsexed day-old Japanese quail chicks were used in a 42-day growing trail. Chicks were individually wing-banded, weighed and randomly distributed into four experimental groups of similar mean body weight (7.81 ± 0.10 g/chick) of 90 chicks each, which consists of three replicates of 30 chicks each. At 42 days of age, birds were transferred to layer quail cages for a 90-day laying trial.

Experimental diets and treatments

Two starter-grower corn-soybean meal (C-SBM) basal diets were formulated to contain recommended protein and recommended energy (RPRE-diet, 24 % CP and 2900 kcal ME/kg diet) according to **NRC (1994)** and low protein and low energy (LPLE-diet, 22 % CP and 2750 kcal ME /kg diet). Two layer C-SBM diets were formulated to contain 20 % CP and 2900 kcal ME/kg diet (RPRE-diet) and 18 % CP and 2750 kcal ME/kg diet (LPLE-diet). Each of the four basal diets was either unsupplemented or supplemented with 0.2% (2 kg/ton) butyric acid glycerides (BA). Therefore, four experimental treatments were used in both starting-growing and laying periods. Each chick group was fed one of the four experimental diets. The composition and chemical analysis of the experimental diets are shown in Table (1).

Tested materials

Since free butyric acid is characterized by a strong unpleasant, penetrating smell, it is almost impossible to be coped with in the feed manufactory and results in poor intakes of the treated feed. However, using the glycerides of butyric acid in recent years solved the problem. The feed additive used in the present study was a blend of mono-, di- and tri-glycerides of butyric acid with “Baby C4®” commercial name that based on the producer (Silo Company, Italy).

Management

Quail chicks were reared under similar management conditions. Ambient temperature was maintained at 34-36 ^{o}C during the 1st week and was decreased by 4 ^{o}C weekly for the next 3 weeks. During the 5th and 6th week temperature was maintained at 22-24 ^{o}C. Birds were received continuous artificial lighting daily during growing trial and 17 h afterwards. Chicks were fed the starter-grower diets from one day to six week and the layer diets from seven to 19 week of age. Mash feed and clean fresh tap water were provided *ad liblitum*.

Measurements and data collection

Growth performance:

Individual body weight (BW, g) and feed intake (FI, g/bird) were weekly recorded to determine body weight gain (BWG, g) [gain = final weight (g) – initial weight (g)]. Feed conversion ratio (FCR, g feed/g gain), protein conversion ratio (PCR), caloric conversion ratio (CCR) and mortality rate (MR) % were also calculated on weekly basis. Performance index (PI) for the starting-growing period, was calculated according the equation reported by **North (1981)**, PI = [(BW, kg/FC) x 100]. Growth rate (GR) for the starting-growing period was also calculated, GR = [(final BW- initial BW) / 0.5 (initial BW+final BW)] × 100.

Carcass parameters:

At the end of the starting-growing period, 24 birds (3 ♂ + 3 ♀/treatment) with BW similar to the mean were slaughtered to determine carcass characteristics. Obtained criteria were dressing, breast and thighs weights. Abdominal fat was removed from the gizzard and abdominal region and individually weighed for each carcass. Both edible giblets (liver, heart and gizzard) and immune organs (thymus, bursa and spleen) were individually removed, weighed and calculated for each organ as % of live BW.

Microbial count and pH measurement:

Ileum and caecum were removed and ligated at both sides and placed into 50 mL tubes in normal saline and then kept at 4 ^{o}C until used for intestinal sampling. Serial dilutions of collected samples from ileum and caecum contents were made up to fifth dilution with normal saline and microbial count of contents in terms of *Lactobacillus spp., Escherichia coli* and *Salmonella spp.* was enumerated by pour plate method **(Quinn *et al.*, 1992)**. Also, the pH of ileum and caecum contents was directly determined by pH-meter.

Blood serum parameters:

At the time of slaughter test (42 days), individual blood samples from randomly 24 birds (3 ♂+3 ♀/treatment) were taken. Sera were individually separated by centrifugation at 3000 rpm for 10 minutes and stored in vials at –20 ^{o}C for later analysis. Frozen sera were thawed and assayed to determine, on individual bases, some biochemical parameters by using Atomic Absorption Spectrophotometer and suitable commercial diagnostic kits following the same steps as described by manufactures. Serum total protein (TP, g/dl), total lipids (TL, mg/dl), cholesterol (Cho, mg/dl), glucose (Glu, mg/dl), calcium (Ca, mg/dl) and phosphorus (P, mg/dl) were determined.

Egg production traits:

Daily egg number (EN) and egg weight (EW, g) as well as weekly feed intake (FI, g/bird) were recorded. Egg production (EP, %), egg mass (EM, g) and feed conversion ratio (FCR, g feed/g egg) were calculated for each replicate and treatment from 7 to 19 weeks of age.

Egg quality:

Egg quality was assessed in nine eggs/treatment (three eggs/replicate) during the last two days of the 90-day laying period. Egg shape index (ESI) was calculated according the equation reported by **Stadleman (1977)**, [ESI = (egg width/egg length) x 100]. Egg specific gravity (SG, mg/cm^3) was determined using the saline flotation method described by **Hempe *et al.* (1988)**. Haugh units were calculated according to **Haugh (1937)** and shell thickness (ST, mm) was measured by a micrometer as an average of three points (top, medial and base). Egg components in terms of the percentages of egg albumen (Alb, %), yolk (Y, %) and shell (S, %) were also determined.

Chemical analysis:

Experimental diets were analyzed following procedures detailed by the Association of Official Analytical Chemists **(AOAC, 1990)** for dry matter (DM), crude protein (CP), crude fiber (CF) and ether extract (EE). The nitrogen free extract (NFE) was calculated by difference. Metabolizable energy (ME) of experimental diets was calculated considering the ME values of different feed ingredients according to the **Feed Composition Tables for Animal and Poultry Feedstuffs Used in Egypt (2001).**

Statistical analysis:

Obtained data were expressed as means±standard error and statistically analyzed by analysis of variance as a factorial arrangement of 2 × 2 according to **Steel and Torrie (1980)**. Also, the General Linear Method (GLM) procedure of **SPSS (1993)** computer statistical program for MS Windows release 6.0 was used. The significant means were ranked using Duncan's Range Test **(Duncan, 1955)** as outlined by **Obi (1990)**. Statistical significance level was tested at probability of $P \leq 0.05$.

RESULTS AND DISCUSSION

Growth performance:

The mean values of growth performance parameters in terms of body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), protein conversion ratio (PCR) and caloric conversion ratio (CCR) are shown in Table (2).

Apart from BA, it was observed that feeding RPRE-diets resulted in significant increase in BW and BWG and significant decrease in FI as well as significant improvement in FCR, PCR and CCR as compared to LPLE-diets. Aside from diet type, feeding BA-supplemented diets gave significant improvement in BW, BWG, FCR, PCR and CCR as well as significant decrease in FI in comparison to BA-free diets. Supplementing BA to RPRE-diet did not significantly affect BW, BWG, FCR, PCR and CCR, whereas it significantly decreased FI as compared to the corresponding control diet. However, supplementing BA to LPLE-diet significantly improved BW, BWG, FCR, PCR and CCR, whereas it significantly decreased FI as compared to the corresponding control diet.

These results are in agreement with those of **Antongiovanni *et al.* (2007)** who found beneficial effect of organic acids on BWG and FCR. In another study, dietary sodium butyrate supplementation increased BWG and improved FCR **(Zhonghong and Yuming, 2007)**. In addition, **Cave (1984**) found that FI was decreased with dietary propionic acid. The improvement in FCR in case of BA supplementation could be explained by the acidic conditions that make the nutrients more available, which monitors better performance.

Oppositely, these results are in disagreement with those of **Izat *et al.* (1990), Vale *et al.* (2004), Leeson *et al.* (2005)** and **Gunal *et al.* (2006)** who reported that supplementation of an organic acid did not have any effect on BWG and FCR. Other results have shown that butyrate had no effect on BW and BWG (**Leeson *et al.*, 2005)**. In addition, **Denli *et al.*, (2003)** found that BW was not significantly affected by organic acid treatments. Furthermore, **Pinchasov and Jensen (1989)** found that BA did not depress FI.

The observed different effects of BA in this study compared with the results of the other studies may be associated with experimental diets and environmental conditions. Several researchers reported that when chicks were housed in a clean environment, organic acids were unaffected on performance **(Miller, 1987)**.

Performance index, growth rate and mortality rate:

The mean values of performance index (PI), growth rate (GR) and mortality rate (MR) % are given in Table (3).

Apart from BA, it was observed that feeding RPRE-diets resulted in significant increase in PI and GR and significant decrease in MR % as compared to LPLE-diets. Concerning acid effect, feeding BA-supplemented diets gave significant improvement in PI and GR and significant decrease in MR % in comparison to BA-free diets. Supplementing BA to RPRE-diet did not significantly affect PI and GR, whereas it significantly decreased MR % as compared to the corresponding control diet. However, supplementing BA to LPLE-diet significantly improved PI and GR, whereas it significantly decreased MR % as compared to the corresponding control diet.

These results are in agreement with those reported that organic acids have positive effects on poultry growth **(Chaveerach *et al.*, 2004)**. The low MR in case of BA supplementation would suggest that the use of BA was effective as antibiotic in reducing mortalities, and thus it could help to reduce the significant increase in therapeutic antimicrobials administered.

Carcass parameters

Results concerning carcass characteristics at 6 wk of age are presented in Table (4).

Regardless of BA, it was appeared that birds given RPRE-diets had significantly increased the percentages of dressing, breast, thighs and abdominal fat as compared to those fed LPLE-diets. Irrespective of diet type, feeding BA-supplemented diets significantly increased carcass parameters except for abdominal fat % that was significantly decreased in comparison to BA-free diets. Adding BA to RPRE-diet did not significantly affect dressing %, breast %, thighs % and abdominal fat as compared to the corresponding BA-free diet. However, supplementing BA to LPLE-diet significantly increased the percentages of dressing, breast and thighs, but it significantly decreased abdominal fat % as compared to the corresponding BA-free diet.

Similarly to the findings of the present study, **Leeson *et al.* (2005)** reported higher carcass yield in broilers fed dietary butyrate. Though no information on literature is available about butyrate effect on abdominal fat in broilers, **Izat *et al.* (1990)** reported significant reduction in abdominal fat content in male broiler chickens by dietary propionic acid supplementation. Thus, it can inferred that dietary organic acid supplementation not only maintains performance but also led to higher carcass yield.

Edible giblets and lymphoid organs:

The percentages of edible giblets in terms of liver**,** heart and gizzard as well as lymphoid organs in terms of thymus, bursa and spleen at 6 wk of age are presented in Table (5).

Regarding edible giblets data, it was indicated that regardless of BA, feeding RPRE-diets significantly increased liver %, heart % and gizzard % as compared to LPLE-diet**.** Regardless of diet type, BA supplementation caused significant increase in edible giblets in comparison to BA-free diets. Supplementing BA to RPRE-diet did not significantly affect edible giblets as compared to the corresponding BA-free diet. However, supplementing BA to LPLE-diet significantly increased edible giblets as compared to the corresponding BA-free diet.

Concerning lymphoid organs results, it was noticed that either diet type or BA showed no significant effect, except for thymus gland that had significantly increased by BA supplementation. Adding BA to RPRE-diet did not significantly affect on lymphoid organs % as compared to the corresponding BA-free diet. However, supplementing BA to LPLE-diet did not significantly affect lymphoid organs %, but it significantly increased thymus as compared to the corresponding BA-free diet.

Similary to the findings of the present study, **Antongiovanni *et al.* (2007)** reported that dietary addition of mixed triglyceride of butyric acid increased liver weight at slaughter and **Shaiful Islam (2005)** observed that absolute weights of bursa and spleen in broilers were not affected by humic acid addition to diets. These lymphoid organs have been chosen as rough indicators for potential effects of the BA on the immune system. Thymus is a good indicator of immune function. **Shelat *et al*. (1997)** revealed that thymus size is a sensitive indicator of health and acute or chronic stress response.

The present results are in disagreement with those that have shown that liver % was not significantly affected by organic acids (**Denli *et al*. 2003)**.

Intestinal microbial count:

The mean of *Lactobacillus spp., Escherichia coli* and *Salmonella spp.* counts in both ileum and caecum contents are given in Table (6).

Regardless of BA, Feeding on RPRE-diets caused no significant effect on the count of *Lactobacillus spp., Escherichia coli* and *Salmonella spp.* in both ileum and caecum contents as compared to LPLE-diet. Irrespective of diet type, BA supplementation resulted in significant increase in *Lactobacillus spp.* count and significant decrease in both *Lactobacillus spp.* and *Escherichia coli* counts in both ileum and caecum contents in comparison to BA-free diets. Supplementing BA to RPRE- or LPLE-diets resulted in significant increase in *Lactobacillus spp.* count and significant decrease in both *Lactobacillus spp.* and *Escherichia coli* counts in both ileum and caecum contents in comparison to the corresponding BA-free diet.

These results are in an agreement with those of **Cox *et al.* (1994)** who found that BA, in particular, was effective in reducing *Salmonella* colonization of the intestine and **Van Immerseel *et al.* (2004a,b)** who significantly indicated reduced levels of *Salmonella* in the ceca of birds fed organic acids. They also reported that butyrate reduces virulence gene expression and invasiveness in *Salmonella enteritidis* leaded to decrease caecal colonization. The effectiveness of BA in reducing the E. coli numbers in the present study may be due to its effect in maintaining a lower pH-value, which is unsuitable for the growth of the organism

The growth promoting effect of BA on beneficial bacteria (*Lactobacilli*) and its growth inhibiting effect on harmful bacteria (*Salmonella spp., E. coli*) is beneficial in regulating intestinal microecological balance. The benefit of this is that BA can confer protection against potential enteropathogenic bacteria, and prevent or cure intestinal diseases. Most importantly, supplementing poultry feed with BA can control the fecal shedding of *Salmonella spp.*, which is a major cause of human foodborne infections following consumption of poultry products.

Intestinal pH:

The mean of intestinal pH values in both ileum and caecum contents are given in Table (7).

Regardless of BA, feeding RPRE-diets caused no significant effect on intestinal pH in both ileum and caecum contents as compared to LPLE-diet Irrespective of diet type, BA supplementation resulted in significant decrease in intestinal pH-value in both ileum and caecum contents as compared to BA-free diets. Supplementing BA to RPRE- or LPLE-diets resulted in significant decrease in intestinal pH-value in both ileum and caecum contents in comparison to the corresponding BA-free diet.

The reduced pH is conducive for the growth of favourable bacteria simultaneously inhibiting the growth of pathogenic bacteria, which grow at a relatively higher pH.

These results are in disagreement with those of **Thompson and Hinton (1997)** who showed that intestinal pH was not affected by formic and propionic acids**.** The acidic pH allows establishment of microorganisms, particularly *Lactobacillus spp***.** **(Sarra *et al.*, 1985)** and prevents E. coli growth and these conditions make the absorptive area more beneficial **(Dofing and Gottschal, 1997),** thereby promoting birds growth.

Blood serum parameters:

Results concerning serum total protein (TP), total lipids (TL), cholesterol (Cho), glucose (Glu), calcium (Ca) and phosphorus (P) concentrations are shown in Table (8).

There were no significant differences in either Cho or Glu among different treatments. Irrespective of BA, feeding RPRE-diets caused significant increase in TP, TL, Ca and P concentrations as compared to LPLE-diet. Regardless of diet type, BA supplementation resulted in significant increase in TP, TL, Ca and P concentrations in comparison to BA-free diets. Supplementing BA to RPRE-diet gave similar TP and TL values as compared to the corresponding BA-free diet, but it significantly increased Ca and P concentrations as compared to the corresponding control diet. However, adding BA to LPLE-diet caused significant increase in TP, TL, Ca and P concentrations as compared to the corresponding control diet.

The non-significant difference in serum level of Cho and Glu agrees with those reported by **Hernandez *et al.* (2006)**, which showed that dietary organic acids had no significant effect on serum levels of Cho and Glu.

The increased serum level of Ca and P with dietary BA is in consistence to previous observations in chickens **(Brenes *et al.*, 2003; Rafacz-Livingston *et al.*, 2005b)**. In contrast, **Hernandez *et al.* (2006)** reported no significant effect of dietary formic acid on serum level of Ca and P. This may be due to the favorable environment in intestinal tract due to BA feeding, which might have helped to digest and absorb more Ca and P.

Two mechanisms for the enhanced mineral serum concentration following BA feeding could be proposed. First, this organic acid might decrease the pH of the digesta in the small intestine that in turn inhibit phytic acid chelate minerals and form insoluble phytate salts which are resistant to hydrolysis by endogenous phytase **(Applegate *et al.*, 2003 and Rafacz-Livingston *et al.*, 2005a).** Another possible mode of action may be associated with chelating effects of organic acids reducing the binding of Ca to phytate that in turn prevent the formation of insoluble Ca-phytate complexes **(Boling *et al.*, 2000; Rafacz-Livingston *et al.*, 2005a**).

Laying performance:

Results concerning laying performance in terms of feed intake (FI), egg production (EP) %, egg number (EN), egg weight (EW), egg mass (EM) and feed conversion ratio (FCR) values are shown in Table (9).

Irrespective of BA, RPRE-diets caused significant improvement in EP %, EN, EW, EM and FCR as well as significant decrease in FI as compared to LPLE-diet. Regardless of diet type, BA supplementation caused significant improvement in EP %, EN, EW, EM and FCR as well as significant decrease in FI in comparison to BA-free diets. Supplementing BA to RPRE-diet gave similar EP %, EN, EW and EM values as compared to the corresponding BA-free diet. The only two exceptions were for FI that was significantly decreased and FCR that was significantly decreased as compared to the corresponding BA-free diet. On the other hand, supplementing BA to LPLE-diet caused significantly improvement in EP %, EW, EM and FCR except for FI that was significantly decreased as compared to the BA-free diet.

Similar to the findings of the present study, **Langhout and Sus (2005)** observed heavier eggs with organic acids supplementation. Contrary to the findings of the present study, **Gama *et al.* (2000)** and **Yesilbag and Colpan (2006)** indicated that the addition of different organic acids in laying hens diet had no effect on the egg weight of laying hens.

These results also are in harmony with those of **Abdel-Rahman (1993)**; **Shrivastav *et al.*, (1993); Zanaty *et al.* (2001); Yakout *et al.* (2004)** and **Garcia *et al.*, (2005)** who reported that EP, EW, EM and FCR were improved with increasing dietary CP level. However, they reported that FI was not significantly affected by dietary CP level.

Egg quality:

Data regarding egg quality in terms of egg shape index (ESI), egg specific gravity (SG), Haugh units (HU), shell thickness (STh), egg albumen (Alb) %, yolk (Y) % and shell (S) % are presented in Table (10).

There were no significant differences in SG among different treatments. Regardless of BA, feeding RPRE-diets caused significant increase in ESI, HU, STh, Y % and S %; but it caused significant decrease in Alb % as compared to LPLE-diet. Irrespective of diet type, BA supplementation caused significant increase in ESI, HU, Y % and S %; but it caused significant decrease in Alb % in comparison to BA-free diets. Supplementing BA to RPRE-diet gave similar ESI, HU, STh, Y % and S % as compared to the corresponding BA-free diet; except for Alb % that was significantly decreased. However, supplementing BA to LPLE-diet caused significant increase in ESI, HU, STh, Y % and S % as well as significant decrease in Alb % as compared to the corresponding BA-free diet.

Incorporation of organic acids in diet attributed significant increase in STh, which may be the consequence of the increased mineral absorption. Similar trend reported by **Soltan (2008)** who found improved STh on organic acids supplementation. Conversely, **Yesilbag and Colpan (2006)** that reported organic acid mixture did not improve STh. Oppositely, the results are not in harmony with those of **Yesilbag and Colpan (2006)** who reported that supplementation of organic acid mixture in laying hens diet were not improved STh. This may be attributed to that organic acid mixture of their trail not including butyrate, which plays a role in the development of intestinal epithelium more than other organic acids **(Pryde *et al.*, 2002; Bron *et al.*, 2002)**.

Similarly, **Yakout *et al.* (2004)** and **Zanaty (2006)** found that STh was significantly increased with increasing CP. This may be due to the increase in EW or the enhancing of Ca deposition in the shell matrix. The present results are also in harmony with the previous researches that showed that increasing CP level increased Y % **(Yakout *et al.*, 2004)** and reduced Alb % **(Akbar *et al.*, 1983)**. On the contrary, **Garcia *et al.* (2005)** reported that dietary CP levels had no effect on Y % and **Zanaty (2006)** reported that increasing CP level decreased Y %.

The results are in harmony with those reported by **Yesilbag and Colpan (2006)** who indicated that supplementation of organic acid mixture in laying hens diet were not improved shell thickness

In conclusion, butyric acid glycerides was effective in maintaining body weight gain and reducing E. coli numbers and found superior for feed conversion ratio. Several additional effects that go beyond these results such as higher carcass yield and low abdominal fat content were also observed by dietary addition of butyric acid glycerides. Based on results of the present study, it can be concluded that using dietary butyric acid glycerides at a level of 0.2% (2 kg/ton) in Japanese quail diets containing sub-optimal energy and protein levels helped in reducing pathogens microflora count, and in turn, improving the health and growth performance of quail and increase economic benefits for farmers.

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

Ingredients	Percentage (%)			
	Starter-grower basal diets*		Layer basal diets*	
	RPRE-diet	LPLE-diet	RPRE-diet	LPLE-diet
Yellow Corn, ground	55.00	54.21	58.00	55.30
Soybean meal (44% CP)	33.51	34.60	25.40	25.00
Corn gluten meal (62% CP)	8.14	3.00	7.03	2.90
Wheat bran	0.00	5.00	0.00	6.73
Vegetable oil	0.00	0.00	1.26	1.73
Dicalcium phosphate	1.58	1.58	2.30	2.26
Limestone	0.81	0.81	5.01	5.02
Common salt (NaCl)	0.34	0.34	0.34	0.34
Premix**	0.30	0.30	0.30	0.30
DL-Methionine	0.03	0.00	0.05	0.11
L-Lysine	0.13	0.00	0.15	0.15
Choline chloride	0.16	0.16	0.16	0.16
Total	100.00	100.00	100.00	100.00
Determined values (%)				
CP %	24.03	22.05	20.00	18.05
CF %	3.44	4.27	3.43	3.90
Calculated values***				
ME (kcal/kg)	2903	2763	2884	2759
Ca %	0.80	0.80	2.50	2.50
Av. Phosphorus %	0.45	0.46	0.55	0.56
L-Lysine %	1.30	1.20	1.10	1.10
DL-Methionine %	0.50	0.46	0.45	0.45
Methionine + Cyst %	0.90	0.91	0.93	0.91

*****Starter-grower and layer basal diets were assigned to 2 levels of Butyric acid {0 & 0.2% (2 kg/ton)}.

**Vitamins and minerals premix provides per kg of diet: 10000 IU vit. A, 11.0 IU vit. E, 1.1 mg vitamin K , 1100 ICU vitamin D3, 5 mg riboflavin, 12 mg Ca pantothenate, 12.1 µg vit. B12, 2.2 mg vit. B6, 2.2 mg thiamin, 44 mg nicotinic acid, 250 mg choline chloride, 1.55 mg folic acid, 0 .11 mg d-biotin, 60 mg Mn, 50 mg Zn, 0.3mg I , 0. 1 mg Co, 30 mg Fe, 5 mg Cu and 1 mg Se.

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

Table2

Table (3): Effect of dietary treatments on performance index, growth rate and mortality rate of Japanese quail during the period from 0 – 6 weeks of age.

Items / Treatments (24% CP)	PI	GR	MR (%)
Energy effects			
RPRE-diet	9.69±0.07[a]	186.63±0.28[a]	3.89±0.06[b]
LPLE-diet	8.34±0.11[b]	185.92±0.61[b]	5.56±0.04[a]
Acid effects			
Butyric acid (0.0kg/kg diet)	8.31±0.08[b]	185.86±0.90[b]	7.78±0.05[a]
Butyric acid (0.2kg/kg diet)	9.72±0.10[a]	186.68±0.54[a]	1.67±0.08[b]
Interaction			
RPRE-diet x 0.0	9.63±0.09[a]	186.75±0.39[a]	6.67±005[b]
RPRE-diet x 0.2	9.74±0.05[a]	186.50±0.47[a]	1.11±0.03[d]
LPLE-diet x 0.0	6.98±0.05[b]	184.97±0.23[b]	8.89±0.07[a]
LPLE-diet x 0.2	9.69±0.10[a]	186.86±0.54[a]	2.22±0.03[c]

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

PI = Performance index GR = Growth rate MR = mortality rate

RPRE-diet = recommended protein and recommended energy

LPLE-diet = low protein and low energy

Table (4): Effect of dietary treatments on carcass characteristics of Japanese quail at 6 weeks of age.

Items / Treatments(24%CP)	(% of BW)			
	Dressing*	Breast	Thighs	Abdominal fat
Energy effects				
RPRE-diet	71.58±1.45[a]	36.53±0.66[a]	25.19±0.14[a]	1.36±0.16[a]
LPLE-diet	69.72±1.62[b]	35.38±0.48[b]	24.09±0.31[b]	0.98±0.09[b]
Acid effects				
Butyric acid (0.0kg/kg diet)	69.66±1.50[b]	35.50±0.51[b]	24.14±0.22[b]	1.31±0.12[a]
Butyric acid (0.2kg/kg diet)	71.64 ±1.27[a]	36.40±0.03[a]	25.14±0.40[a]	1.03±0.10[b]
Interaction				
RPRE-diet x 0.0	71.54±1.28[a]	36.44±0.62[a]	25.13±0.51[a]	1.44±0.13[a]
RPRE-diet x 0.2	71.62±1.15[a]	36.61±0.51[a]	25.25±0.12[a]	1.28±0.18[b]
LPLE-diet x 0.0	67.78±1.29[b]	34.56±0.65[b]	23.15±0.13[b]	1.18±0.15[b]
LPLE-diet x 0.2	71.65±1.24[a]	36.19±0.50[a]	25.03±0.09[a]	0.77±0.14[c]

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

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

RPRE-diet = recommended protein and recommended energy

LPLE-diet = low protein and low energy

Table (5): Effect of dietary treatments on edible giblets and immune organs% of Japanese quail at 6 weeks of age.

Items	Edible giblets (%)			Immune organs (%)		
Treatments (24% CP)	Liver (%)	Heart (%)	Gizzard (%)	Thymus (%)	Bursa (%)	Spleen (%)
Energy effects						
RPRE-diet	3.24±0.14[a]	1.26±0.13[a]	2.75±0.09[a]	0.18±0.01[a]	0.09±0.11	0.05±0.02
LPLE-diet	2.74±0.10[b]	1.12±0.10[b]	2.57±0.10[b]	0.14±0.11[b]	0.09±0.01	0.06±0.01
Acid effects						
Butyric acid (0.0kg/kg diet)	2.71±0.19[b]	1.12±0.14[b]	2.58±0.13[b]	0.14±0.02[b]	0.07±0.03	0.05±0.03
Butyric acid (0.2kg/kg diet)	3.27±0.10[a]	1.26±0.11[a]	2.74±0.15[a]	0.17±0.01[a]	0.11±0.02	0.06±0.02
Interaction						
RPRE-diet x 0.0	3.26±0.21[a]	1.26±0.14[a]	2.77±0.16[a]	0.19±0.02[a]	0.08±0.02	0.05±0.11
RPRE-diet x 0.2	3.21±0.17[a]	1.25±0.12[a]	2.73±0.14[a]	0.17±0.05[a]	0.09±0.03	0.05±0.02
LPLE-diet x 0.0	2.15±0.22[c]	0.97±0.10[b]	2.39±0.16[b]	0.09±0.03[b]	0.05±0.01	0.05±0.05
LPLE-diet x 0.2	3.32±0.18[b]	1.27±0.11[a]	2.75±0.12[a]	0.18±0.02[a]	0.12±0.05	0.07±0.03

Means in the same column within the same effect having different letters are significantly Different at $P \leq 0.05$.

RPRE-diet = recommended protein and recommended energy

LPLE-diet = low protein and low energy

Table (6): Effect of dietary treatments on microbiota counts in the ileum and caecum of Japanese quail at 6 weeks of age.

Items Treatments (24% CP)	Microflora count ($\log_{10}$ CFU/g ileum or caecum content)					
	Ileum			Caecum		
	Lactobacillus spp.	***Escherichia coli***	***Salmonella spp.***	***Lactobacillus spp.***	***Escherichia coli***	***Salmonella spp.***
Energy effects						
RPRE-diet	5.12±0.08	12.31±0.13	2.81±0.14	20.06±0.12	29.43±1.18	4.94±1.06
LPLE-diet	6.08±0.13	12.48±0.11	2.82±0.07	20.03±0.05	29.12±1.22	4.93±1.08
Acid effects						
Butyric acid (0.0kg/kg diet)	4.38±0.05[b]	14.63±0.12[a]	3.51±0.10[a]	18.11±0.14[b]	34.63±1.15[a]	6.63±1.11[a]
Butyric acid (0.2kg/kg diet)	6.82±0.10[a]	10.16±0.06[b]	2.12±0.11[b]	21.98±0.10[a]	23.92±1.20[b]	3.24±1.00[b]
Interaction						
RPRE-diet x 0.0	4.42±0.21[b]	14.50±0.31[a]	3.47±0.04[a]	18.22±0.08[b]	35.02±1.21[a]	6.59±1.03[a]
RPRE-diet x 0.2	6.82±0.12[a]	10.12±0.14[b]	2.14±0.06[b]	21.90±0.02[a]	23.83±1.06[b]	3.28±1.05[b]
LPLE-diet x 0.0	4.34±0.11[b]	14.75±0.11[a]	3.54±0.03[a]	18.00±0.05[b]	34.23±1.12[a]	6.66±1.04[a]
LPLE-diet x 0.2	7.02±0.10[a]	10.20±0.06[b]	2.09±0.10[b]	22.05±0.02[a]	24.00±1.14[b]	3.20±1.10[b]

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

RPRE-diet = recommended protein and recommended energy

LPLE-diet = low protein and low energy

Table (7): Effect of dietary treatments on pH in the ileum and caecum of Japanese quail at 6 weeks of age.

Items / Treatments (24% CP)	Ileum	Caecum
Energy effects		
RPRE-diet	7.00±0.20	6.82±0.15
LPLE-diet	7.04±0.14	6.73±0.23
Acid effects		
Butyric acid (0.0kg/kg diet)	7.39±0.19[a]	7.09±0.24[a]
Butyric acid (0.2kg/kg diet)	6.65±0.17[b]	6.46±0.18[b]
Interaction		
RPRE-diet x 0.0	7.40±0.21[a]	7.04±0.22[a]
RPRE-diet x 0.2	6.67±0.24[b]	6.41±0.13[b]
LPLE-diet x 0.0	7.38±0.11[a]	7.13±0.17[a]
LPLE-diet x 0.2	6.62±0.19[b]	6.50±0.18[b]

Means in the same column within the same effect having different letters are significantly different at $P \leq 0.05$.
RPRE-diet = recommended protein and recommended energy
LPLE-diet = low protein and low energy

Table (8): Effect of dietary treatments on some serum blood parameters of Japanese quail at 6 weeks of age.

Items / Treatments (20% CP)	TP (g/dl)	TL (mg/dl)	Cho (mg/dl)	Glu (mg/dl)	Ca (mg/dl)	P (mg/dl)
Energy effects						
RPRE-diet	4.18±0.12[a]	6.89±0.03[a]	274.5±3.91	256±3.76	10.12±0.03[a]	4.34±0.02[a]
LPLE-diet	3.97±0.13[b]	6.48±0.03[b]	263.0±3.77	244±3.63	9.09±0.01[b]	3.97±0.01[b]
Acid effects						
Butyric acid (0.0kg/kg diet)	4.00±0.11[b]	6.50±0.01[b]	272.5±3.22	254±3.33	8.95±0.01[b]	3.88±0.01[b]
Butyric acid (0.2kg/kg diet)	4.16±0.10[a]	6.87±0.01[a]	265.0±3.50	246±3.37	10.26±0.02[a]	4.68±0.02[a]
Interaction						
RPRE-diet x 0.0	4.17±0.10[a]	6.87±0.02[a]	274.0±4.63	259±4.05	9.67±0.01[b]	4.00±0.01[b]
RPRE-diet x 0.2	4.19±0.08[a]	6.91±0.01[a]	271.0±4.06	253±4.84	10.57±0.01[a]	4.68±0.02[a]
LPLE-diet x 0.0	3.82±0.10[b]	6.12±0.03[b]	267.0±4.10	249±4.90	8.22±0.02[c]	3.75±0.01[c]
LPLE-diet x 0.2	4.12±0.06[a]	6.83±0.02[a]	260.0±4.34	239±4.10	9.95±0.01[b]	4.19±0.01[b]

Means in the same column within the same effect having different letters are significantly different at $P \leq 0.05$.
TP = Total protein TL = Total lipids Cho = cholesterol Glu = Glucose Ca = Calcium P = Phosphorus
RPRE-diet = recommended protein and recommended energy
LPLE-diet = low protein and low energy

Table (9): Effect of dietary treatments on performance of laying Japanese quail from 7 to 19 weeks of age.

Items / Treatments (20% CP)	FI (g/hen/day)	EP (%)	EN (No./hen/day)	EW (g)	EM (g/hen/day)	FCR (g feed/g egg)
Energy effects						
RPRE-diet	23.48±0.05^{b}	81.70±1.19^{a}	0.82±0.02^{a}	11.22±0.01^{a}	9.15±0.01^{a}	2.57±0.02^{b}
LPLE-diet	25.03±0.10^{a}	77.19±1.41^{b}	0.77±0.01^{b}	10.94±0.01^{b}	8.45±0.02^{b}	2.99±0.01^{a}
Acid effects						
Butyric acid (0.0kg/kg diet)	25.87±0.17^{a}	77.36±1.30^{b}	0.77±0.01^{b}	10.95±0.02^{b}	8.46±0.01^{b}	3.08±0.02^{a}
Butyric acid (0.2kg/kg diet)	22.64±0.10^{b}	81.53±1.15^{a}	0.82±0.01^{a}	11.21±0.02^{a}	9.14±0.02^{a}	2.48±0.02^{b}
Interaction						
RPRE-diet x 0.0	24.71±0.19^{b}	81.26±1.20^{a}	0.81±0.02^{a}	11.16±0.01^{a}	9.04±0.01^{a}	2.73±0.02^{a}
RPRE-diet x 0.2	22.24±0.31^{c}	82.14±1.14^{a}	0.82±0.01^{a}	11.27±0.01^{a}	9.26±0.01^{a}	2.40±0.01^{b}
LPLE-diet x 0.0	27.02±0.23^{a}	73.46±1.32^{b}	0.73±0.02^{b}	10.73±0.02^{b}	7.88±0.02^{b}	3.43±0.02^{a}
LPLE-diet x 0.2	23.04±0.14^{c}	80.91±1.54^{a}	0.81±0.01^{a}	11.14±0.01^{a}	9.02±0.01^{a}	2.55±0.02^{b}

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

FI = Feed intake EP = Egg production EN = Egg number EW = Egg weight
EM = Egg mass FCR = Feed conversion ratio
RPRE-diet = recommended protein and recommended energy
LPLE-diet = low protein and low energy

Table 10

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تأثير استخدام حمض البيوتريك على أداء كتاكيت السمان اليابانى المغذى على مستويات مثلى وتحت المثلى من الطاقة والبروتين.

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أستخدم فى هذه الدراسة ٣٦٠ كتكوت سمان يابانى غير مجنس عمر يوم تم توزيعها بالتساوى على ٤ معاملات غذائية (٣ مكررات/معاملة) استمرت حتى عمر ١٩ أسبوع وذلك بهدف معرفة تأثير إضافة حمض البيوتريك على أداء النمو وبعض صفات الذبيحة ومقاييس الدم وكذلك أداء إنتاج البيض للسمان اليابانى.

تم تكوين عليقتين نمو (كنترول) **الأولى** تحتوى على ٢٤٪ بروتين خام و٢٩٠٠ كيلو كالورى طاقة ممثلة/كجم علف لتغطى الاحتياجات الغذائية للسمان اليابانى طبقاً للمجلس القومى الأمريكى للبحوث لسنة ١٩٩٤ **والثانية** تحتوى على ٢٢٪ بروتين خام و٢٧٥٠ كيلو كالورى طاقة ممثلة/كجم علف لتغذية الكتاكيت خلال فترة النمو (١-٤٢ يوم). تم تكوين عليقتين بياض (كنترول) **الأولى** تحتوى على ٢٠٪ بروتين خام و٢٩٠٠ كيلو كالورى طاقة ممثلة/كجم علف لتغطى الاحتياجات الغذائية للسمان اليابانى طبقاً للمجلس القومى الأمريكى للبحوث لسنة ١٩٩٤ **والثانية** تحتوى على ١٨٪ بروتين خام و٢٧٥٠ كيلو كالورى طاقة ممثلة/كجم علف وذلك لتغذية الطيور خلال فترة إنتاج البيض (٧-١٩ أسبوع). تم إضافة أو عدم إضافة ٢كجم/طن علف حمض البيوتريك إلى علائق النمو والبياض الكنترول، وبذلك يكون هناك ٤ معاملات غذائية فى كل من فترة النمو وإنتاج البيض.

فى نهاية فترة النمو (عمر ٤٢ يوم) تم ذبح ٢٤ طائر (٣ إناث+٣ ذكور/معاملة) (أنثى+ ذكر/مكرر) لتقدير صفات الذبيحة. وفى فترة إنتاج البيض (٧-١٩ أسبوع) تم تسجيل عدد ووزن وكتلة البيض ومعدل إنتاج البيض والغذاء المأكول ومعدل تحويل الغذاء، كما تم تكسير عدد ١٠ بيضات من كل معاملة فى نهاية فترة الـ ٩٠ يوم من إنتاج البيض لتقدير جودة البيض.

أوضحت النتائج خلال فترة النمو أن إضافة حمض البيوتريك للعليقة المحتوية على مستوى البروتين والطاقة طبقاً للـ NRC أدى لنقص معنوى فى كلا من العلف المأكول، دهن البطن، أعداد بكتيريا إيشيريشيا كولا والسالمونيلا فى اللفائفى والأعور، وزيادة معنوية فى كلا من أعداد بكتريا اللاكتوباسليس ودرجة الـ pH فى اللفائفى والأعور، الكالسيوم والفوسفور فى سيرم الدم.

بينما أدى إضافة حمض البيوتريك للعليقة المنخفضة فى البروتين والطاقة إلى تحسن معظم صفات النمو وأعضاء المناعة، وزيادة معنوية فى أعداد بكتريا اللاكتوباسليس ودرجة الـ pH تحسناً معنوياً فى صفات سيرم الدم ، بينما لم يؤثر ذلك على العلف المأكول وغدة البيرسا والطحال.

كما أوضحت النتائج خلال فترة إنتاج البيض أن إضافة حمض البيوتريك للعليقة المحتوية على مستوى البروتين والطاقة طبقاً للـ NRC أدى لنقص معنوى فى العلف المأكول وتحسن معنوى فى معامل تحويل الغذاء وزيادة معنوية فى نسبة بياض البيض، بينما أدى إضافة حمض البيوتريك للعليقة المنخفضة فى البروتين والطاقة إلى تحسن كلا من معظم صفات إنتاج البيض، دليل شكل البيضة، وحدة هاو، سمك القشرة، نسبة الصفار، ونسبة القشرة.

من وجهة النظر الغذائية يمكن أن يستنتج من نتائج هذه الدراسة - تحت ظروف التجربة الحالية - أن إضافة حمض البيوتريك بمعدل ٢كجم/طن علف إلى علائق السمان اليابانى المحتوية على مستوى البروتين والطاقة طبقاً للـ NRC ساعدت فى تقليل أعداد الكائنات الحية الدقيقة الضارة الموجودة بأمعاء الطيور وهذا بدوره يحسن أداء ومناعة الطيور.

الكلمات الدالة: (حمض البيوتريك – البيوتيرات - أداء – سيرم الدم - إنتاج البيض– جودة البيضة - السمان).