

Effects of Organic Acids Supplement on Performance and Gut Parameters in Male Japanese quail (*Coturnix Coturnix*)

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ABSTRACT: This experiment was conducted to evaluate usage organic acids supplement (acetic, lactic and butyric acids), in the basal diet and their effects on performance and gastrointestinal parameters on male Japanese quails in 35 day old period. Performance parameters include live body weight (LBW), body weight gain (BWG), feed intake (FI), and feed efficiency ratio (FCR), carcass, breast and thigh weight. Gastrointestinal parameters include the along of gastrointestinal tract (GIT), intestine weight, intestinal villus and crypt in male Japanese quail. A total of 560 Japanese quail were randomly divided in to 7 experimental treatments with 4 replicates and arranged in a completely randomized design. The experimental period lasted 5 weeks and during this period. Experimental diets consisted of: basal diet without organic acids supplement (T1), basal diet with 125 mg/kg acetic acid supplement (T2), basal diet with 105 mg/kg lactic acid supplement (T3), basal diet with 112 mg/kg butyric acid supplement (T4), basal diet with 62.5 mg/kg acetic acid and 52.5 mg/kg lactic acid supplement (T5), basal diet with 62.5 mg/kg acetic acid and 56 mg/kg butyric acid supplement (T6) and basal diet with 52.5 mg/kg lactic acid and 56 mg/kg butyric acid supplement (T7). Results for performance analyses showed the treatment contain of the lactic acid (T3), butyric acid (T4) and acetic acid (T2) alone, significantly increased the LBW ($P<0.0001$), WG ($P<0.0001$) and decreased FCR respectively ($P<0.0005$). Also, results showed the treatment contain of the lactic acid (T3) and butyric acid (T4) alone, respectively increased the FI significantly ($P<0.0001$). Carcass, breast and thigh weights, not significantly affected with the all of the experiment treatment, but treatment contain of the lactic acid (T3), have been increased numerically in these parameters. In the other hand, treatments contains of the butyric acid (T4), acetic acid and butyric acid (T6) and lactic acid and butyric acid (T7), respectively increased the intestinal weight significantly ($P<0.0001$). Furthermore, treatments contain butyric acid (T4), lactic acid and butyric acid (T7), lactic acid (T3) and acetic acid and butyric acid (T6), respectively increased the intestinal villi length in the jejunum significantly ($P<0.0005$). In addition, treatments contain acetic acid and butyric acid (T6), lactic acid (T3) and butyric acid (T4) respectively increased the intestinal crypt in the jejunum significantly ($P<0.0001$). Also, treatment contain lactic acid (T3) significantly decreased the GIT in Japanese quail ($P<0.0001$). In conclude the lactic acid even alone and too mid another organic acid improved the performance parameters in male Japanese quail at 35 days old. Also carcass, breast and thigh weights not significantly affected with all of the treatment. In the other hand butyric acid even alone and too mid another organic acid improved the intestinal parameters.

Key words: Carcass, crypt, gastrointestinal, Japanese quail, microflora and villi.

INTRODUCTION

The removal antibiotic growth promoters from poultry diets necessitate search for natural alternatives as replacements in growing flocks, especially under conditions of average management and quality. Most supplements that are used as alternatives to antibiotics in poultry production such as plant materials or extracts such as herbs, essential oils, probiotics, prebiotics and organic acids, have effects on microflora, either directly or indirectly (Claiford, 1999). Gram positive bacteria are generally more sensitive to organic acids than gram-negative bacteria (Claiford, 1999).

Because of the growing concern over the transmission and proliferation of resistant bacteria via the food chain, the European Union (EU) in 2006 banned antibiotic growth promoters to be used as additives in animal nutrition. So there are used the need for alternative strategies to minimize the risk of spreading antibiotic resistance from animals to humans via food chain. The alternative which has showed some potential in this regard are organic acids. Organic acids and their salts are Generally Regarded as Safe (GRAS) and have been approved by most member states of the EU to be used as feed additives in animal production (Gornowicz *et al.*, 2002; Dibner *et al.*, 2005; Cakir *et al.*, 2008).

Organic acids have growth promoting properties and can be used as alternatives to antibiotics (Patten and Waldroup 1988). The addition of organic acids to the broiler diet reduces the production of toxic components by bacteria and the colonization of pathogens in the GIT (Denli *et al.*, 2003). Organic acids may affect the integrity of microbial cell membrane or cell macromolecules or interfere with nutrient transport and energy metabolism causing bactericidal effect (Ricke, 2003). Following organic acid feeding, reduction in gastric pH occurs which may increase the pepsin activity (Kirchgeßner and Roth, 1982) and the peptides arising from pepsin proteolysis trigger the release of hormones, including gastric and cholecystokinin, which regulate the digestion and absorption of protein (Kirchgeßner and Roth, 1982; Hersey, 1987). Organic acids supplementation have been reported to decrease colonization of pathogens and production of toxic metabolites, improve digestibility of protein and minerals like calcium, phosphorus, magnesium and zinc and also serve as substrates in the intermediary metabolism (Kirchgeßner and Roth, 1988). The more of the previous study was carried on with the objectives to determine the effects of organic acids supplementation on the growth performance and intestinal microflora of broiler chickens. But, the aim of the present study was to evaluate the efficiency of organic acids supplementation as growth promoters in Japanese quail. In the other hand, in this study the alone effect of organic acids was estimated the performance and gastrointestinal parameters on the male Japanese quail. Japanese quails has a lab animal (Pointer *et al.*, 2009) and this subject on the Japanese quail not carried out, so the aims of this study are the measured amounts of the growth rate, yield performance factors, gastrointestinal microflora and absorption parameters in GIT such as intestinal crypt depth and villi in Japanese quail with consumption of dissimilar organic acids supplement in diets. About quail in this subject not the similar research, so this experimental is an initiatory research and it has seem results in this study was interesting. In this study, alone effect and interaction effects of the organic acids were estimated on Japanese quail.

MATERIALS AND METHODS

A. Animals and diets

A total of 560 one-day old Japanese quail chicks of from male and female sex were placed in 12 pens of 1.4×0.6 meters with twenty birds per each pen. Feed and water were provided ad libitum. After 20 days of the starting of experiment, male quails were separated from the female and arranged in to the treatment. The organic acids used in this experiment, include the acetic acid (pKa = 4.76, Purity = 80% and Density = 1.05 g/cm³), lactic acid (pKa = 3.86, Purity = 95% and

Density = 1.21 g/cm³) and butyric acid (pKa = 4.82, Purity = 90% and Density = 0.99 g/ml). The experimental design consisted in a completely randomized design with 7 treatments T1) basal diet without organic acids supplement, T2) basal diet with 125 mg/kg acetic acid supplement, T3) basal diet with 105 mg/kg lactic acid supplement, T4) basal diet with 112 mg/kg butyric acid, T5) basal diet with 62.5 mg/kg acetic acid + 52.5 mg/kg lactic acid, T6) basal diet with 62.5 mg/kg acetic acid + 56 mg/kg butyric acid and T7) basal diet with 52.5 mg/kg lactic acid + 56 mg/kg butyric acid, with four replicates. The experimental diets of were isonitrogenous and isoenergetic. All of the diets balanced for the DCAB and kept about in 250 (meq/kg). Diets were formulated by adding acetic acid (80%), lactic acid (95%) and butyric acid (90%) be based diet (corn and soybean meal) that met requirement recommended by the National Research Council (1994). Diets of balanced with the UFFDA software. The control diet was not enriched with organic acids supplement and was administered throughout the 21 days of experimental period (starter). The organic acids supplements were replaced with corn in diets during 2 different periods (grower and finisher). Crude protein levels and metabolisable energy was formulated with NRC (1994) recommendation. These diets (Table 1) were formulated to meet nutrient requirements according to NRC (1994). Diets were containing the same levels of methionine, lysine, vitamins and minerals. The treatment diets of were isoenergetic and isonitrogenous. Experimental diets include the three different organic acids such as:

The Japanese quails were weighted at the start of the experimental period, live body weight and total feed intake were recorded and feed conversion ratio was calculated at each week of the experiment. Mortality was also recorded for each treatment. Ingredient composition and nutrient analysis for each treatment is described in Table 2.

B. Collection of samples

In the end of the experiment period at 35 day, four male Japanese quail from each replicate were selected. The birds slaughtered after weighting and the live body weight (LBW) was measured. Feed intake was determined the end of the each day. Afterward, feed conversion ratio (FCR) was calculated. BWG was determined during at 21 - 35 days old. In the end of the experiment at 35 day, male Japanese quail from each replicate were slaughtered after weighting and gastrointestinal track was evicted and with sodium chloride dilution (155 m/mol) for dispel the excretion has been washed. In the other hand, The 10 gram of the intestinal liquids was collection in vials then addition the formalin (10%) in the vial for fixation the tissue and immediately transfers to microbiological lab to determine the *E. coli* and *salmonella* population.

Calculated nutrient content

ME kcal/kg	2900	2900	2900	2900	2900	2900	2900
Crude protein (%)	20	20	20	20	20	20	20
Calcium (%)	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Available P (%)	0.33	0.33	0.33	0.33	0.33	0.33	0.33
Met (%)	0.78	0.78	0.78	0.78	0.78	0.78	0.78
Met + Cys (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Lys (%)	1	1	1	1	1	1	1
Tryp (%)	0.33	0.33	0.33	0.33	0.33	0.33	0.33
acetic acid (%)	0	1	0	0	0.05	0.05	0
lactic acid (%)	0	0	1	0	0.05	0	0.05
butyric acid (%)	0	0	0	1	0	0.05	0.05
ME/CP	145	145	145	145	145	145	145
Ca/P	7.14	7.14	7.14	7.14	7.14	7.14	7.14
DCAB (meq/kg)	250.11	250.11	250.11	250.11	250.11	250.11	250.11

Vitamin content of diets provided per kilogram of diet: vitamin A, D, E and K. Composition of mineral premix provided as follows per kilogram of premix: Mn, 120,000 mg; Zn, 80,000 mg; Fe, 90,000 mg; Cu, 15,000 mg; I, 1,600 mg; Se, 500 mg; Co, 600 mg.

C. Samples analyses

In the histological lab 2 cm of the jejunum has been amputated, then fixation with buffer formalin dilution (10 %, pH = 7.4). After the fixation levels, samples put the solid paraffin and cut the attenuate sheet with microtome. Since the samples be paint and this samples were noted in histological study to determine the intestinal villi and crypt depth (Liu *et al.*, 2010). In the other hand, in microbial laboratory one gram of the intestine liquids was taken and transition to the saline dilution (0.9 normal). After the assimilated, samples were incubated in 38°C for the 48 hour. After these process under the optical microscope were determined the *E. coli* and *Salmonella* population (Liu *et al.*, 2010).

D. Statistical analyses

Data were analyzed in a complete randomized design using the GLM procedure of SAS version 12 (SAS Inst. Inc., Cary, NC).

$$Y_j = \mu + i + ij$$

Where:

Y_j = All dependent variable

μ = Overall mean

i = The fixes effect of organic acids levels ($i = 1, 2, 3$)

ij = The random effect of residual

Duncan multiple ranges (Duncan, 1955) used to compare means.

RESULTS**A. Performance parameters**

Result for performance parameters analyses shown in table 3. Result showed the treatments contain which lactic acid (T3), butyric acid (T4) and acetic acid (T2) respectively have highest effect on the LBW and BWG as significantly increased LBW between another treatments in male Japanese quail ($P < 0.0001$). In

addition, result showed the treatments contain which lactic acid (T3), lactic acid plus butyric acid (T7), acetic acid (T2) and butyric acid (T4) respectively have highest effect on the FI and significantly increased FI between another treatments ($P < 0.0001$). Result showed the treatments contain which lactic acid (T3), butyric acid (T4) and acetic acid (T2) respectively have highest effect on the FCR and significantly decreased FCR between another treatments in male Japanese quail ($P < 0.0005$). These results were according with some researchers finding (Ao *et al.*, 2009; Mohamed and Bahnas, 2009; Abdel-Fattah *et al.*, 2008; Lesson *et al.*, 2005; Nuh Ocak *et al.*, 2009; Moharrey, 2005; Denli *et al.*, 2003; Fushimi *et al.*, 2001; Adil *et al.*, 2011). Result showed that with usage organic acid supplements in experimental diet, the LBW significantly increased and from 142.25 gram for treatment without organic acids supplement reached to 159.25, 160.21 and 169.17 grams respectively for treatments contains acetic acid (T2), butyric acid (T4) and lactic acid (T3) ($P < 0.0001$). Also, result showed that with usage organic acid supplements in experimental diet, the BWG significantly increased and from 91.25 and 91.59 gram for treatment without organic acids supplement and treatment contain acetic acid plus butyric acid (T6) reached to 105.58, 100.25 and 99.62 grams respectively for treatments contains lactic acid (T3), butyric acid (T4) and acetic acid (T2) ($P < 0.0001$). Also, with usage organic acids supplement in experimental diets, the FI significantly increased and from 551.70 gram for treatment without organic acids supplement reached to 569.25, 559.18, 555.25 and 552.00 gram respectively for treatments contains lactic acid (T3), lactic acid plus butyric acid (T7), acetic acid (T2) and butyric acid (T4) ($P < 0.0001$).

In addition, result showed the treatments contain lactic acid (T3), butyric acid (T4) and acetic acid (T2) respectively decreased FCR from 3.88 for treatment without organic acids supplement reached to 3.36, 3.44 and 3.48 respectively for treatments contains lactic acid (T3), butyric acid (T4) and acetic acid (T2) ($P < 0.0005$).

B. Characteristics analyses

Result for carcass characteristics analyses shown in table 4. Result for production parts parameters showed the all of the treatment not significantly affected on the carcass, breast and thigh weights. But treatment contain of the lactic acid (T3), have been increased numerically in these production parameters.

Table 3: Effects of organic acids supplement on performance in male Japanese quail on 35 days old.

Treatments	Treatments							SE M	P value
	T1	T2	T3	T4	T5	T6	T7		
Live Body Weight (g)	142.25 ^d	159.25 ^b	169.17 ^a	160.21 ^b	158.19 ^{bc}	143.18 ^{cd}	157.18 ^{bc}	1.91	0.0001
Body Weight Gain (g)	91.25 ^c	99.62 ^{ab}	105.58 ^a	100.25 ^{ab}	98.00 ^{abc}	91.59 ^c	98.59 ^{bc}	1.05	0.0001
Feed Intake (g)	551.70 ^{cd}	555.25 ^{bc}	569.25 ^a	552.05 ^{cd}	553.05 ^{bcd}	549.17 ^d	559.18 ^b	1.87	0.0001
Feed Conversion Ratio	3.88 ^a	3.48 ^{bc}	3.36 ^c	3.44 ^{bc}	3.49 ^{bc}	3.83 ^{ab}	3.55 ^b	1.91	0.0185

Means in rows with no common superscript differ significantly ($P < 0.05$).

[(T1, basal diet without organic acids supplement), (T2, basal diet with 125 mg/kg acetic acid supplement), (T3, basal diet with 105 mg/kg lactic acid supplement), (T4, basal diet with 112 mg/kg butyric acid), (T5, basal diet with 62.5 mg/kg acetic acid + 52.5 mg/kg lactic acid), (T6, basal diet with 62.5 mg/kg acetic acid + 56 mg/kg butyric acid) and (T7, basal diet with 52.5 mg/kg lactic acid + 56 mg/kg butyric acid)]

Table 4: Effects of organic acids supplement on carcass characteristics in male Japanese quail on 35 days old.

Treatments	Treatments							SE M	P value
	T1	T2	T3	T4	T5	T6	T7		
Carcass weight (%)	71.50	75.51	79.28	73.18	74.12	72.31	75.50	0.30	0.1171
Breast weight (%)	32.50	33.35	35.81	34.12	35.15	33.80	33.90	0.75	0.2151
Thigh weight (%)	19.87	20.51	21.87	21.23	20.21	20.48	20.91	0.56	0.0821

Means in rows with no common superscript differ significantly ($P < 0.05$).

[(T1, basal diet without organic acids supplement), (T2, basal diet with 125 mg/kg acetic acid supplement), (T3, basal diet with 105 mg/kg lactic acid supplement), (T4, basal diet with 112 mg/kg butyric acid), (T5, basal diet with 62.5 mg/kg acetic acid + 52.5 mg/kg lactic acid), (T6, basal diet with 62.5 mg/kg acetic acid + 56 mg/kg butyric acid) and (T7, basal diet with 52.5 mg/kg lactic acid + 56 mg/kg butyric acid)]

C. Gastrointestinal tract parameters

Result for intestinal parameters analyses shown in table 5. Result showed the treatments contain which lactic acid (T3) and acetic acid (T2) respectively have highest effect on the gut size. Treatments contain lactic acid (T3) and acetic acid (T2) significantly decreased gastrointestinal track length between another treatments in male Japanese quail ($P < 0.0001$). Therewith, result showed that with usage organic acid supplements in experimental diet, the gut length significantly decreased and 34.85 centimeter for treatment without organic acids supplement reached to 30.80 and 31.43 Cm respectively for treatments contains lactic acid (T3) and acetic acid (T2). Also result showed the treatments contain which butyric acid (T4) between another treatment contain the organic acids has a highest effect on the intestine weight in

male Japanese quail as increased intestine weight significantly ($P < 0.0001$), whereas result showed that with usage organic acid supplements in experimental diet, the intestine weight significantly increased from 3.21 gram for treatment without organic acids supplement reached to 4.31 gram for treatments contains butyric acid (T4). These results were according with some researchers finding (Denbove, 2000; Gunal *et al.*, 2006; Garcia *et al.*, 2007). Furthermore, it has noticeable interaction effects between acetic acid and lactic acid with butyric acid so the since treatment contain butyric acid (T4), treatment contain acetic acid plus butyric acid (T6) and lactic acid plus butyric acid (T7) have a highest effect on the intestine weight significantly ($P < 0.0001$). With better attention between all of the treatments contain the butyric acid significantly increased the intestine weight.

Examination of jejunum epithelium by light microscopic of semi thin section from male Japanese quail exposed to 35 day old, result showed marked morphological changes in intestinal villi and crypt depth so treatment contain butyric acid (T4) has a highest effect on intestinal villi and crypt depth so increased significantly into another treatments contain organic acid ($P < 0.0005$ and $P < 0.0001$, respectively). More attention between all of the treatments contain the butyric acid significantly increased the intestine villi and crypt depth such as treatment contain acetic acid plus butyric acid (T6) and lactic acid plus butyric acid (T7). In continues, result showed with usage organic acids supplements in experimental diets, the intestinal villi significantly increased from 89.50 μm for treatment without organic

acids supplement reached to 133.81, 132.90 and 131.18 μm respectively for treatments contains lactic acid (T4), acetic acid plus butyric acid (T7) and acetic acid plus butyric acid (T6). Also, data showed, with usage organic acids supplements in experimental diets, the intestinal crypt depth significantly increased from 51.25 micrometer for treatment without organic acids supplement reached respectively to 75.51, 74.80 and 74.51 μm for treatments contains lactic acid plus butyric acid (T7) and acetic acid plus butyric acid (T6). These results were according with some researchers finding (Miles *et al.*, 2006; Sheikh *et al.*, 2010), with handling the gastrointestinal bacteria population, examination of jejunum paunch by light microscopic from male Japanese quail exposed to 35 day old.

Table 5: Effects of organic acids supplement on morphology and microflora population of gastrointestinal tract in male Japanese quail on 35 days old.

Treatments	T1	T2	T3	T4	T5	T6	T7	SE	P
								M	value
Gut Length (cm)	34.85 ^{ab}	31.43 ^{cd}	30.80 ^d	32.51 ^{bc}	35.50 ^a	32.81 ^{bc}	31.52 ^{cd}	1.13	0.0001
Intestine Weight (%)	3.21 ^d	3.99 ^{cd}	4.18 ^{abc}	4.31 ^a	4.12 ^{bc}	4.20 ^{ab}	4.20 ^{ab}	0.05	0.0001
Intestinal Villi Height (μm)	89.50 ^e	115.25 ^d	130.15 ^{bc}	133.81 ^a	129.81 ^c	131.18 ^{bc}	132.90 ^{ab}	24.8	0.0251
Intestinal Crypt Depth (μm)	51.25 ^e	72.31 ^{bc}	72.32 ^{bc}	75.51 ^a	67.25 ^d	74.51 ^{ab}	74.80 ^{ab}	2.60	0.0001

Means in rows with no common superscript differ significantly ($P < 0.05$).

[(T1, basal diet without organic acids supplement), (T2, basal diet with 125 mg/kg acetic acid supplement), (T3, basal diet with 105 mg/kg lactic acid supplement), (T4, basal diet with 112 mg/kg butyric acid), (T5, basal diet with 62.5 mg/kg acetic acid + 52.5 mg/kg lactic acid), (T6, basal diet with 62.5 mg/kg acetic acid + 56 mg/kg butyric acid) and (T7, basal diet with 52.5 mg/kg lactic acid + 56 mg/kg butyric acid)]

DISCUSSION

In marginal study has been reported the organic acids not significantly effects on the performance parameters in Japanese quail (Cave, 1984). In contrast, the result of same studies has been showed the some organic acids such as Propionic acid, lactic acid and acetic acid have a significantly effect on the performance parameters in Japanese quail (Fushimi *et al.*, 2001; Denli *et al.*, 2003; Moharrey, 2005; Lesson *et al.*, 2005; Abdel-Fattah *et al.*, 2008; Ao *et al.*, 2009; Mohamed and Bahnas, 2009; Nuh Ocak *et al.*, 2009; Adil *et al.*, 2011;). In the other hand, this research results have been shown highest effect of lactic acid on the increase the LBW, BWG and FI and improved the FCR in 35 days old Japanese quail. Whitherward, treatments of the organic acids not significantly effect on the yield parameters, but treatments contain the lactic acid numerically improvement the carcass, breast and thigh weight but no significant. Lactic acid has a bottommost pKa (3.86) between the acetic acid (pKa = 4.76) and butyric acid (pKa = 4.82), has a maximum acidity power toward another organic acids it used in this study (Samanta and

Ghosh, 2008). Therefore, it has seemed the lactic acid were limited the pathogen microorganisms in the feed and GIT (Apajalahti *et al.*, 2004; Garrido *et al.*, 2004; Bourassa *et al.*, 2005; Moharrey and Mahzonieh, 2005; Harris *et al.*, 2006; Laury *et al.*, 2009; Hassan *et al.*, 2010). In addition it has seemed the lactic acid decreased the pathogen microorganisms in crop and decreases the competition for nutrient between the quails and bacteria. In finally with decrease the competition for nutrient between the quails and microorganism, digestibility of the feeds and absorption the nutrient has improved in the gastrointestinal tract. Whitherward, some researches was reported the lactic acid is palatable for birds (Apajalahti *et al.*, 2004; Garrido *et al.*, 2004; Bourassa *et al.*, 2005; Moharrey and Mahzonieh, 2005; Harris *et al.*, 2006; Laury *et al.*, 2009; Hassan *et al.*, 2010), so it has presumably this feature of the lactic acid author the increase of the feed intake in this trial and this confederation is a causation the increase LBW and BWG. Mid improved the live body weight and feed intake, FCR has an improvement impress the treatments contain the lactic acid.

In addition, result has been showed; the treatments contain of butyric acid have a highest significantly effects on the increase the intestinal villi height and crypt depth in male Japanese quail at 35 days old. It has seemed the butyric acid has a not catalysis in above zone of the GIT such as crop, proventriculos and gizzard and his effects has survived until the intestines so this leads to increase intestinal villi height and crypt depth in male Japanese quail. In the other hand, increase the intestinal villi height and crypt depth lead to significantly increase the total weight of the GIT as the all of the experimental treatment contain the butyric acid causation improved the GIT weight in this trial. In the other hand, it has seemed the butyric acid in this experiment with increase the secretion of gastrin hormone and this gnomon has a simulations roles on the intestinal villi height and crypt depth development (Apajalahti *et al.*, 2004; Garrido *et al.*, 2004; Bourassa *et al.*, 2005; Moharrery and Mahzonieh, 2005; Harris *et al.*, 2006; Laury *et al.*, 2009; Hassan *et al.*, 2010). In issue, it has seemed the lactic acid alone in comparison with the acetic acid and butyric acid has positive effects on the performance parameters and carcass characteristic in Japanese quail. In conclude the lactic acid even alone and too mid another organic acid improved the performance parameters in male Japanese quail at 35 days old. Also carcass, breast and thigh weights not significantly affected with all of the treatment. In the other hand butyric acid even alone and too mid another organic acid improved the intestinal parameters.

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