Research Article

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The effect of organic acid, zeolite, or their combination on performance, some serum indices, and ileum pH values in broilers fed with different phosphorus levels

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Abstract: The aim of this study was to investigate the effects of the addition of organic acid (OA), zeolite (ZE), or both to broiler diets with microbial phytase containing low and adequate levels of phosphorus (P) on performance, some serum indices, and ileum pH values. For the study, 480 broiler chicks 1-day old were assigned equally to 8 groups, with 6 replicates (10 chicks per replicate) carried out over 42 days. A basal diet containing 600 FTU of phytase enzyme was formulated separately for the starter and grower periods. Experimental groups were divided into 2 main groups: low P [(0.39% P; basal diet (B), B+OA, B+ZE, and B+OA+ZE)] and adequate P [(0.70% P; basal diet (B), B+OA, B+ZE, and B+OA+ZE)]. The average body weight of the low-P groups was significantly lower than that of the adequate-P groups. However, birds fed ZE and OA+ZE had increased body weights. Within the first 21 days of the study, the addition of ZE and OA+ZE to diets significantly decreased feed intake, and feed conversion rates improved depending on the P level and the OA+ZE addition. Serum P levels increased depending on the decrease in the level of P in the diet at 21 days, and the ALP concentration at day 42 was found to be statistically lower in the group fed an OA+ZE diet. Ileum pH values in the OA group were found to be lower than those in other groups at 42 days. These results proved that the addition of ZE and OA+ZE to the diet with microbial phytase containing low and adequate levels of P had positive effects on performance.

Key words: Broiler, organic acid, zeolite, performance, phosphorus

Farklı fosfor düzeyi içeren broyler rasyonlarına organik asit, zeolit veya kombinasyonları ilavesinin performans, bazı serum parametreleri ile ileum pH değerlerine etkisi

Özet: Bu çalışma; organik asit (OA), zeolit ve her ikisinin birlikte düşük ve yeterli düzeyde fosfor (P) içeren mikrobiyal fitaz katkılı rasyona ilavesinin broyler performansı, bazı serum parametreleri ve ileum pH değeri üzerine etkilerini araştırmak için yapılmıştır. 480 adet günlük broyler civcivler eşit olarak sekiz gruba bölünmüştür. 600 FTU fitaz enzimi içeren bazal rasyon başlangıç ve büyütme dönemleri için ayrı olarak hazırlanmıştır. Deneme grupları düşük P [(% 0,39 P; bazal rasyon (B), B+OA, B+ZE ve B+OA+ZE)] ve yeterli P [(% 0,70 P; bazal rasyon (B), B+OA, B+ZE and B+OA+ZE)] düzeyi ile beslenen iki ana gruba ayrılmıştır. Düşük-P grubunun ortalama canlı ağırlığı yeterli-P

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grubundan önemli derecede düşük bulunmuştur. Ayrıca ZE ve OA+ZE ilavesi canlı ağırlıkta artışa neden olmuştur. Çalışmanın 0-21. günleri arasında ZE ve OA+ZE ilavesi yem tüketimini önemli derecede azaltmış ve rasyonun P düzeyi ile OA+ZE ilavesine bağlı olarak yemden yararlanma oranı iyileşmiştir. Denemenin 21. gününde rasyonun P düzeyindeki azalmaya bağlı olarak serum P düzeyi artmış, 42. gündeki ALP konsantrasyonu ise OA+ZE ilavesi yapılan grupta önemli derecede düşük bulunmuştur. OA grubunun ileum pH değerinin 42. günde diğer gruplardan daha düşük olduğu tespit edilmiştir. Sonuçlar düşük ve yeterli düzeyde P içeren mikrobiyal fitaz katkılı rasyonlara ZE ve OA+ZE kombinasyonunun ilavesinin performans üzerine olumlu etki yarattığını göstermiştir.

Anahtar sözcükler: Broyler, organik asit, zeolit, performans, fosfor

Introduction

Poultry's requirements for calcium (Ca) and phosphorus (P), which are 2 of the most important macroelements, are mainly supplied by grains and inorganic resources. However, utilization of plant-originated P by animals is very limited because 60%-80% of the P in plants is found in the form of phytate-P. In addition to limiting the utilization of P, phytate also limits utilization of some other essential nutrients (1-3). Addition of the phytase enzyme to the diet of monogastric animals is an effective and practical method for both utilization of phytate-P and reduction of P disposal, which is a serious concern due to environmental pollution (4). Increasing the utilization of dietary P improves the performance and retention of mineral substances. For example, performance criteria of broiler chicks, including body weight gain, feed consumption, and feed conversion rate, are significantly improved by non-phytate-P and the addition of phytase (1,3,5,6). Broilers fed with phytase-supplemented diets gained more weight, and there was a positive correlation between phytase content and non-phytate-P content (4). Qian et al. (7) suggested that the phytase enzyme and Ca-to-tP ratio are important factors in degrading phytate and improving phytate-P and Ca utilization in broiler chickens.

A few studies have suggested that organic acid (OA) may improve phytate-P utilization in monogastric animals. Such treatment also reduced the usable P requirement by 0.1% and increased the utilization of P without affecting calcium needs or utilization of calcium (8). Boling et al. (9) reported that citric acid improved phytate-P utilization by competitively chelating Ca, reducing the formation of insoluble Ca-phytate complexes. Boling-Frankenbach et al. (8) reported that the effect of OA on the intestinal pH was positive but that this effect was more limited than expected, since OAs are rapidly metabolized in the intestine. The level of pH in avian GI tracts has been reported to facilitate the formation of a phytate-protein complex; thus, such a complex cannot be hydrolyzed by the added phytase enzyme (10).

Another feed additive that affects Ca and P utilization is zeolite (ZE). ZEs have been used in animal feeds as adsorbents due to their ability to absorb toxic agents. However, their effects on nutrients are questionable. Eliot and Edwards (11) reported that aluminum silicate, a chemical present in the structure of ZEs, reacted with P, resulting in the formation of a complex that is difficult to digest and therefore decreasing the utilization of P. They also reported that natural ZEs improved the feed conversion rate but did not affect other parameters. Chung and Baker (12) reported that ZEs did not have any limiting effect on the utilization of P. The objectives of this study were as follows: 1) to evaluate the effects of OA, ZE, and OA+ZE on growth performance, serum mineral concentration and alkaline phosphatase (ALP) activities, and ileum pH levels; and 2) to evaluate effects and interactions of OA, ZE, and OA+ZE in broiler chickens fed diets with microbial phytase and different P levels.

Materials and methods

The experiment was conducted under appropriate animal care. A total of 480 1-day-old broiler chicks (Ross 308 strain) were obtained from a commercial hatchery. The birds were housed in an environmentally controlled room. The fluorescent lights were on for 24 h each day. Diets in mash form and water were provided for ad libitum consumption. Chicks were assigned equally to 8 groups, each group containing 10 chicks, to receive 8 dietary treatments of wheat-soybean-based diets for 6 replicates of each treatment. Diets were formulated by considering the nutrient requirements of broilers for starter and grower periods (13) (Table 1). The treatment structure consisted of 2 levels of P, 0.39% and 0.70%. Phytase was added to the basal diets. Experimental groups were divided into 2 main groups, low P [(0.39% P; basal diet (B), B+OA, B+ZE, and B+OA+ZE)] and adequate P [(0.70% P; basal diet (B), B+OA, B+ZE, and B+OA+ZE)]. As feed additives, 600 FTU/kg of phytase enzyme [fermented substance from Aspergillus oryzae (Natuphos 500, 500,000 FTU/kg phytase, BASF Corp.)], 20 g/kg of organic acid (Salb Curb, Kemin Industries, Inc.; a mold inhibitor for processed feed ingredients and animal feeds containing primarily propionic acid), and 20 g/kg of natural zeolite (containing 98% clinoptilolite) were used in the experimental diets.

At days 21 and 42 of the experimental period, the chicks were weighed and feed intake was recorded for the feed conversion rate. At the end of each experimental period (day 21 or day 42), 2 chicks from each subgroup were randomly selected. Blood samples were taken from the wing vein for subsequent determination of minerals (Ca and P) and alkaline phosphatase in the serum. Blood was centrifuged and serum was stored at −20 °C until analysis. After the birds were killed by cervical dislocation, the small intestine was removed and ileal contents, from Meckel's diverticulum to 40 mm proximal to the ileocecal junction, were collected. Fresh samples of ileal digesta were taken to determine pH.

Diets were analyzed for crude protein (CP) and dry matter (by drying in an oven at 105 °C for

Table 1. Ingredient and nutrient composition (%) of broiler starter and grower diets.

Items	Starter (days 0-21)		Grower (days 22-42)	
	Low P	Adequate P	Low P	Adequate P
Feed stuffs Wheat	56.55	54.05	59.55	59.55
Soybean meal	34.15	35.65	30.15	30.15
Wheat bran	1.00	-	2.00	-
Sunflower oil	6.00	6.00	6.00	6.00
Salt	0.40	0.40	0.40	0.40
Dicalcium phosphate	-	2.00	-	2.00
Limestone	1.10	1.10	1.10	1.10
DL-Methionine	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10
Vit.-Min. Premix[1]	0.50	0.50	0.50	0.50
Nutritional content (data on dry matter)				
ME, kcal/kg	3183	3120	3201	3157
CP	23.00	23.10	21.40	21.10
Lysine	1.20	1.20	1.10	1.10
Met+Cys	0.90	0.90	0.80	0.80
Ca	0.55	1.10	0.53	1.10
P	0.39	0.70	0.39	0.70

[1]Premix/kg: Vitamin A, 10,000,000 IU; vitamin D_3, 1,500,000 IU; vitamin E, 40,000 mg; vitamin K_3, 3000 mg; vitamin B_1, 2200 mg; vitamin B_2, 4500 mg; niacin, 30,000 mg; Cal.D-Pant.,13,000 mg; vitamin B_6, 3000 mg; vitamin B_{12}, 15 mg; folic acid, 1500 mg; biotin, 100 mg; choline chloride, 250,000 mg; vitamin C, 12,000 mg; Mn, 80,000 mg; Zn, 60,000 mg; Fe, 30,000 mg; Cu, 5000 mg; I, 1000 mg; Co, 200 mg; Se, 150 mg.

10 h) by the methods of the AOAC (14). Ca and P contents of diets were determined as colorimetric and spectrophotometric values, respectively (14).

A factorial arrangement of treatment was used to investigate the effect of the 2 levels of P and 4 diets. The statistical model included P levels and diets as main effects. Data were evaluated for analysis of variance using the general linear models in SPSS (15). Mean differences were determined by Duncan's multiple range test.

Results

In groups fed with the microbial phytase-supplemented basal diet with an adequate P level, improved body weight was seen, compared to the diet with low P (P < 0.01) (Table 2). Only OA+ZE had a significant effect on the body weight of the animals (P < 0.05). Average body weights at days 21 and 42 were 651.68 and 2033.94 g in the low-P diet group, respectively, and the corresponding values were 667.97 and 2117.53 g in the adequate-P diet

Table 2. Effect of P levels and OA and/or ZE on body weight (BW), average feed intake (AFI), and FCR of broilers from days 0 to 42.

	P levels (%)	BW, g		AFI, g			FCR (g/g)		
		Days 0-21	Days 22-42	Days 0-21	Days 22-42	Days 0-42	Days 0-21	Days 22-42	Days 0-42
Groups									
Control	0.39	650.00[cd]	2016.38[d]	977.28[ab]	2603.76	3581.14	1.62[c]	1.92[b]	1.81[a]
OA	0.39	653.05[cd]	1992.89[d]	971.21[ab]	2491.21	3462.43	1.60[c]	1.89[cb]	1.78[ab]
ZE	0.39	639.16[e]	2065.00[c]	951.33[b]	2474.17	3425.50	1.60[c]	1.74[c]	1.69[bc]
OA+ZE	0.39	664.75[bcd]	2089.78[bc]	966.34[b]	2564.27	3530.61	1.56[c]	1.82[c]	1.73[bc]
Control	0.70	673.90[bc]	2098.18[bc]	995.64[a]	2535.98	3531.62	1.58[c]	1.79[c]	1.72[bc]
OA	0.70	670.66[bc]	2123.75[ab]	974.67[ab]	2476.87	3451.54	1.56[bc]	1.73[c]	1.66[c]
ZE	0.70	680.83[ab]	2116.17[ab]	975.75[ab]	2532.65	3508.45	1.51[ab]	1.94[a]	1.69[bc]
OA+ZE	0.70	703.16[a]	2160.44[a]	977.62[ab]	2505.42	3483.04	1.49[a]	1.74[c]	1.65[c]
SEM		3.20	5.91	3.11	15.54	16.99	0.09	0.24	0.013
P levels									
0.39%		651.68[b]	2033.94[b]	966.54[b]	2533.35	3499.89	1.59[a]	1.84	1.75[a]
0.70%		667.97[a]	2117.53[a]	980.92[a]	2512.73	3493.66	1.53[b]	1.80	1.68[b]
Diet									
Control		661.94[b]	2048.22[b]	986.46[a]	2569.86	3556.33	1.60[a]	1.86	1.77[a]
OA		661.86[b]	2050.29[b]	972.94[ab]	2484.04	3456.98	1.57[a]	1.81	1.72[ab]
ZE		664.70[b]	2098.18[a]	963.54[b]	2503.41	3466.97	1.55[ab]	1.84	1.69[b]
OA+ZE		683.62[a]	2115.26[a]	971.98[ab]	2534.84	3506.82	1.52[b]	1.78	1.68[b]
Source of variation					Probability				
P Levels		0.01	0.01	0.02	NS	NS	0.01	NS	0.01
Diet		0.05	0.05	0.01	NS	NS	0.01	NS	0.05
P X Diet		0.05	0.001	0.05	NS	NS	0.004	NS	NS

OA: organic acid, ZE: zeolite, P: phosphorus

[a,b,c] within columns: means with no common letter differ significantly (P < 0.05)

NS = Nonsignificant (P > 0.05)

group (P < 0.01). At 42 days, the positive effect of an OA+ZE addition on body weight continued (P < 0.05). Average feed consumption levels during the first 21 days of the study varied depending on the level of P (P < 0.02).

The results for blood parameters between days 21 and 42 are provided in Table 3. Serum P levels had increased depending on the decrease in the level of P in the diet at day 21, and the ALP concentration on day 42 was found to be statistically lower in the group fed an OA+ZE diet (P < 0.05). The differences between serum Ca levels were not statistically significant.

Effect of OA and/or ZE addition to the diets containing microbial phytase and low or adequate P on the pH level of ileum contents was evaluated (Table 3). There was a difference between the groups at 42 days as a result of the addition of AO and ZE to the diet and their interactions (P < 0.001).

Discussion

In this study, adequate levels of P in the diet more positively affected growth performance than diets with a low level of P (P < 0.05). This finding is in agreement with the results of studies in which

Table 3. Effects of P levels and OA and/or ZE on serum indices and ileum pH of broilers.

	P levels (%)	Ca, mg/dL		P, mg/dL		ALP, mg/dL		Ileum pH	
		Days 0-21	Days 22-42	Days 0-21	Days 22-42	Days 0-21	Days 22-42	Days 0-21	Days 22-42
Groups									
Control	0.39	10.96	10.33	6.84	7.27	154.25	228.83^{ab}	6.37	6.64^{a}
OA	0.39	10.55	10.58	6.99	7.06	138.00	283.33^{a}	6.28	6.44^{b}
ZE	0.39	9.63	9.52	6.10	6.86	133.58	203.08^{abc}	6.41	6.35^{bc}
OA+ZE	0.39	10.08	10.10	6.55	6.58	148.75	190.00^{ab}	6.37	6.63^{a}
Control	0.70	10.27	10.21	6.88	6.89	166.25	238.58^{ab}	6.39	6.48^{b}
OA	0.70	10.24	9.87	6.42	6.42	106.17	226.58^{ab}	6.32	6.23^{c}
ZE	0.70	9.66	9.77	6.04	5.98	136.25	166.50^{bc}	6.33	6.70^{a}
OA+ZE	0.70	10.06	9.88	6.27	6.32	114.92	156.08^{c}	6.45	6.45^{b}
SEM		0.14	0.10	0.11	0.12	4.72	7.49	0.022	0.021
P levels									
0.39%		10.30	9.95	6.94^{a}	6.94^{a}	143.65	226.31	6.35	6.52
0.70%		10.05	9.93	6.40^{b}	6.40^{b}	130.90	196.94	6.37	6.46
Diet									
Control		10.62	10.26	6.85^{a}	7.07	160.25	233.71^{ab}	6.38	6.56^{a}
OA		10.39	10.22	6.70^{ab}	6.73	122.08	254.96^{a}	6.30	6.33^{b}
ZE		9.87	9.65	6.07^{b}	6.42	134.92	184.79^{ab}	6.37	6.52^{a}
OA+ZE		10.07	9.61	6.40^{ab}	6.45	131.83	173.04^{b}	6.41	6.54^{a}
Source of variation							Probability		
P Levels		NS	NS	0.01	0.01	NS	NS	NS	NS
Diet		NS	NS	0.05	NS	NS	0.05	NS	0.001
P X Diet		NS	NS	NS	NS	NS	0.05	NS	0.001

OA: organic acid, ZE: zeolite, P: phosphorus

[a,b,c] within columns: means with no common letter differ significantly (P < 0.05)

NS = Nonsignificant (P > 0.05)

the positive effect of phytase on growth performance was more evident as the P level in the diet increased (16,17). Payne et al. (6) claimed that a decrease in non-phytate-P (nPP) in a phytase-supplemented broiler diet affected growth performance negatively, compared to the growth performance of animals fed with adequate levels of nPP. In our study, the findings also confirmed that the level of P, whether low or adequate, was one of the main parameters affecting growth performance. On the other hand, Qian et al. (18) reported that the efficiency of microbial phytase was better in the absence of P, or at low levels of P, in broiler or pig diets. In this study, however, the effect of the phytase enzyme in low-P diets was not evident compared to that observed in the adequate-P diet group. Within the first 3 weeks of the study, the addition of ZE and OA+ZE to diets significantly decreased feed intake ($P < 0.01$). In this period, the feed conversion rate improved depending on the P level ($P < 0.01$) and the addition of OA+ZE ($P < 0.01$). It has been determined that, in general, the phytase enzyme improved the performance not only by increasing the feed consumption but also by increasing the feed conversion rate (19). Perney et al. (20) reported that feed consumption increased together with the increase in the level of usable P (0.21%, 0.29%, and 0.37%) in broilers fed with diets containing usable P. On the other hand, the addition of phytase to diets containing 0.29% P did not affect the feed consumption rate. In the same study, it was reported that increasing the level of P in the diet resulted in increased feed consumption, and the addition of phytase did not cause any significant change in feed consumption. This reported finding supports our finding that the addition of phytase enzyme to diets with low or adequate levels of P did not affect the feed consumption within the 42 days of the study period. In the current study, in addition to the microbial phytase, increasing the level of P from 0.39% to 0.70% improved the feed conversion rate within the first 21 days ($P < 0.01$). However, this effect disappeared within the second 21 days. This situation can be explained by findings that different Ca-to-tP ratios of diets, even if phytase was added, did not have a constant effect on the feed conversion rate (21). There are different feed additives improving the efficiency of phytase enzyme in broilers. One of these additives is OA. In the current study, it was determined that the difference in growth performance was dependent on the level of P and the addition of an OA+ZE mixture to the diet. OA alone did not have an effect on feed consumption or body weight. It was concluded that the effect of OA on growth performance was similar to effects of a basal diet. In studies that investigated the OAs, citric acid was used as a source of OA in broiler diets. Citric acid has been reported to increase the utilization of phytate-P (22), thus decreasing the need for P (8). In contrast, in another study, additions of 1-alpha-hydroxycholecalciferol, citric acid, and phytase to the diet increased the utilization of phytate-P and had a positive effect on growth performance (23). Brenes et al. (17) reported a positive interaction between usable P, phytase, OAs, and the feed conversion rate. In this study, a synergistic effect of OA and phytase on the performance was not found. The reason for this can be that the released P could prevent a Ca/P balance, which results in a negative effect on growth performance (17). In addition, it has been thought that the use of an OA mixture rather than a single acid could have inhibited the expected effect.

The current study was undertaken to investigate the interactions between ZE and different feed additives, as well as to evaluate the effects of these on the animals. When the data for live weight, feed consumption, and feed conversion ratios were evaluated, it was observed that the addition of ZE to low-P diets affected performance negatively, compared to adequate-P diets. This situation can be explained by the fact that ZE has a negative effect on P metabolism, or that it has no effect on phytate or P metabolism at all (12). On the other hand, ZE affected the performance positively when the level of P was increased from 0.39% to 0.70% ($P < 0.05$). This finding supports similar findings that ZE addition increases growth performance and improves the feed conversion rate (24). Another interesting finding of the present study is that the combination of OA+ZE additives affected growth performance at both P levels. To our knowledge, there is no published literature about the use of OA and ZE together. However, it has been thought that OA may improve the efficiency of the phytase enzyme as well as reduce the negative effects of ZE on P metabolism. The synergistic effect between ZE and OA probably occurred in the way that ZE provides an opportunity for the nutrients to be absorbed by the GI tract more efficiently by slowing down the passage of ingesta

(25), and OA improves the hybridization of phytate by phytase by acidification of the environment (26).

It was determined that the serum P value was affected at 21 days by the dietary P level ($P < 0.01$). Addition of ZE to the diet at 42 days was determined to decrease serum P levels ($P < 0.05$). When the P values at 21 days were evaluated, it could be seen that they were lower in the low-P group than in the adequate-P group. This finding is contradictory to arguments that the serum P level decreases in animals fed with low-P diets, depending on the retention of P (17). Viveros et al. (16) claimed that the increase in serum P levels was dependent on the increase in the Ca-to-tP ratio in the diet. In our study, it was found that the blood P level was higher in the group with a Ca-to-P ratio of 1.4:1 and a dietary P level of 0.39%, compared to the other group with a Ca-to-P ratio of 1.6:1 and a P level of 0.70%. It was also detected that the added ZE resulted in a decrease in serum P levels at 21 days ($P < 0.05$). It is known that aluminum silicate, present in the structure of ZE, reacts with P and results in the formation of a complex that is difficult to digest, such that ZE suppresses the P utilization while increasing the Ca utilization (11). Ravindran et al. (27) observed an increase in P retention in low-P diets for birds. Our results can be interpreted as showing that ZE additions to the diet cause a negative effect on P metabolism and that the animals respond to this condition by improving the P retention. It was determined that serum ALP levels changed depending on the diet. These changes were not statistically significant at 21 days, but the difference between the OA and OA+ZE groups became significant ($P < 0.05$) at 42 days. ALP is an enzyme playing an important role during the mineralization period. Increase of blood ALP levels as a result of low P levels in the diet is in agreement with the results of similar studies carried out in broilers (17) and in turkeys (28). However, results of one study suggested that the increase in ALP levels in broilers was related to deficiency in bone development or deficiency in liver functions (17). It has been found that the addition of OA+ZE to the diet resulted in a decrease in ALP levels when compared to other treatment groups ($P < 0.05$). Unlike our results, it has been reported that phytase additions decreased the ALP activity, and this decrease was more evident in animals fed with low-P diets (16). In our study, a blood ALP decrease was seen in the adequate-P diet group. It has been reported that such a decrease could be related to the addition of phytase to the diet and may indicate that production of this enzyme decreased as a result of the increase in P utilization (29).

As the pH values of ileum contents, presented in Table 3, were evaluated, there was no difference between groups at 21 days, but significant differences were observed at 42 days as a result of the addition of OA and ZE to the diet and their interactions ($P < 0.01$). pH values in the OA group were found to be lower than those of the other groups ($P < 0.01$). The level of pH in avian GI tracts has been reported to facilitate the formation of a phytate-protein complex; thus, such a complex cannot be hydrolyzed by the added phytase enzyme (10). Boling-Frankenbach et al. (8) reported that the effect of OA on the intestinal pH level was positive, but that this effect was more limited than expected since OAs are rapidly metabolized in the intestine. In our study, however, pH levels in OA-supplemented groups were lower than those in other groups. This could be a result of OA activity. It has been reported that there is a relationship between digestion pH, digestion viscosity, and the density of ingesta. An increase in digestion viscosity reduced the density of ingesta, causing an increase in the pH of intestinal content (30).

Practically, the addition of an OA+ZE combination to the diet with microbial phytase containing low and adequate levels of P had positive effects on performance.

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