# EFFECT OF ADDING DRY YEAST AND FOLIC ACID ON IMPROVING THE PHYSIOLOGICAL AND PRODUCTIVE PERFORMANCE OF QUAIL. <sup>1</sup>Suha A. Rasheed <sup>1</sup>S. M. A. Al Nuaimmi

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#### ABSTRACT

This study was aimed to investigate the different effects of folic acid and/or *saccharomyces cerevisiae* on different aspects of Japanese quails. Eighty quail chicks were used, divided into four groups (20 chicks each), the first group was the control, treated with normal ration and drinking water. The second group was treated with *saccharomyces cerevisiae* 2.5% in the ration, the third group was treated with folic acid (5mg/L. in drinking water), whereas the last group was treated with both folic acid and yeast as stated above. All treatments were for seven weeks, then the birds were sacrificed, and blood was collected to undergo the parameters. Results revealed that the treatment with yeast or folic acid significantly (p<0.05) decreased both RBC and Hb while WBC elevated as a result of the treatment in both males and females. In males, the concentration of cholesterol and triglycerides reduced in all treatment groups, with no effect on triglycerides. Testosterone was elevated in males treated with folic acid and/or yeast.

*Key words:* blood picture, hormones, lipid profile, some biochemical aspects.

المستخلص

تهدف هذه الدراسة إلى التحري عن التأثيرات المختلفة لحمض الفوليك و/أو الخميرة الجافة على الجوانب الفسلجية المختلفة لطائر السمان. استخدم ثمانين طائر من صغار السمان بعمر يوم واحد مقسمة إلى أربع مجاميع (20 طيور لكل مجموعة) المجموعة الأولى تمثلت بمجموعة السيطرة والتي عوملت بالعليقة القياسية ومياه الشرب. المجموعة الثانية عوملت بـ 2.5 ٪ خميرة جافة مع العليقة، فيما عوملت المجموعة الثالثة بحمض الفوليك (5 ملجم / لتر في ماء الشرب)، بينما عوملت المجموعة الأخيرة بكل من حمض الفوليك والخميرة الجافة كما هو مذكور أعلاه. استمرت جميع المعاملات لمدة سبعة أسابيع بعدها تم قتل الطيور وجمع عينات الدم. أوضحت النتائج أن المعاملة بالخميرة أو حمض الفوليك أدت إلى انخفاض معنوي ( P بعدها تم قتل الطيور وجمع عينات الدم. أوضحت النتائج أن المعاملة بالخميرة أو حمض الفوليك أدت إلى انخفاض معنوي ( P بعدها تم قتل الطيور وجمع عينات الدم الموسحت النتائج أن المعاملة بالخميرة أو حمض الفوليك أدت إلى انخفاض معنوي ( P الأولاث. انخفض وزن الجسم نتيجة معاملة طائر السمان بالخميرة و/أو حمض الفوليك في كل من الذكور والإناث. انخفض وزن الجسم نتيجة معاملة طائر السمان بالخميرة و/أو حمض الفوليك في كل من الذكور والإناث. لوحظ في والإناث. انخفض وزن الجسم نتيجة معاملة طائر السمان بالخميرة و/أو حمض الفوليك في كل من الذكور والإناث. لوحظ في والإناث. انخفض وزن الجسم نتيجة معاملة طائر السمان بالخميرة ورأو حمض الفوليك في كل من الذكور والإناث. وحظ في والإناث. انخفض وزن الجسم نتيجة معاملة طائر السمان بالخميرة ورأو حمض الفوليك في كل من الذكور والإناث. لوحظ في والإناث. انخفاض تركيز الكوليسترول والدهون الثلاثية في جميع المجاميع المعاملة مقارنة بمجموعة السيطرة، بينما ارتفع مستوى الموليسترول عند الإناث في مجاميع المعاملة، دون أي تأثير على الدهون الثلاثية. كان هرمون التستوستيرون مرتفع في الذكور الذفاض تركيز الكوليسترول والدهون الثلاثية مومامة، دون أي تأثير على الدهون الثلاثية. كان هرمون التستوستيرون مرتفعا في الذكور الذين عولجوا بحمض الفوليك و/أو الخميرة.

الكلمات المفتاحية: بعض جوانب الكيمياء الحياتية, صورة الدم, مستوى الدهون, هرمونات.

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# INTRODUCTION

The poultries industry is a growing challenge in terms of providing the diet and protection against diseases. Studies have tried to find the best solutions for all problems facing this industry, with a big focus on the economic aspect. Vitamins are essential for all organisms, and an important part of the poultry's rations. Folic acid  $(C_{19}H_{19}N_7O_6)$  is one of the B complex member (B9), stored in the liver (15). Currently, folic acid represents a focus for nutrition researches due to its vital roles, including protection, metabolism, and epigenetics. In addition, folic acid has been recognized as a key factor in hematopoiesis, and the function of blood cells. Folic acid is also crucial in the pathways responsible for the synthesis of both purines and pyrimidines, and consequently the synthesis of nucleic acids (16). Furthermore, folic acid involves amino acids synthesis(17) and cell division and growth(20). Deficiency or disorders in folic acid metabolism cause depletion in 5methyltetrahydrofolate, the active form of folic acid after being ingested and absorbed (1). A wealth of studies highlighted the advantages of folic acid in the poultry diet. It has been found that folic acid reduces fats in both abdomen and liver in the broiler (22). Another study found that dietary folic acid improves spermatozoal mRNA expression in roosters (33). On the other hand, faulty expression in particular genes has been detected in the broiler offspring from maternal folic acid deficiency (34). One of the important sources of probiotics is yeast. Therefore, using yeast may help in improving immunity and body physiology in livestock (24). Saccharomyces cerevisiae is amongst yeast genera which have been used as food additives in animals. The vital cell of S. cerevisiae and/or the extract is rich in the growth and production requirements such as proteins in addition, it represents a source of most B vitamins such as vitamin B6, thiamin, biotin, riboflavin, nicotinic acid and pantothenic acid, minerals are also available in yeast (14). Using saccharomyces cerevisiae as a food additive is efficient interims of detoxifying mycotoxins in the mucous membrane and villi, it also helps in eliminating damages to organs due to the ability to reduce stress (14). Despite the different effects of S.

*cerevisiae* has been subjected to a lot of analysis, there is still a gap regarding their effects on avian performance. Here, we have undertaken a study to investigate the physiological roles of folic acid and/or *saccharomyces cerevisiae* on quail, using different aspects.

# MATERIALS AND METHODS

Animals: Eighty of recently hatched Japanese quail chicks were used in this experiment, kept in the animal house, College of Veterinary Medicine, University of Mosul, under controlled conditions (25). Chicks were fed with primary ration (Yellow corn 30%, wheat 22%, concentrated protein 15%, soybean meal 32%, calcium 0.9%, salt 0.1% ) for three weeks, then with production ration (Yellow corn 40%, wheat 25%, concentrated protein 10%, soybean meal 20%, calcium 4.5%, salt 0.5% ) for another four weeks.

Chicks were randomly divided into four groups (20 chicks/each) as follows:

1- Control: fed on ration and given drinking water *Ad libitum* for 7 weeks.

2- Yeast group: Treated with 2.5% yeast with the ration for 7 weeks.

3- Folic acid group: Treated with folic acid 5mg/L. in drinking water for 7 weeks.

4- Yeast+ folic acid group: Treated with both yeast and folic acid as mentioned above for 7 weeks.

# Specimens

Blood samples were collected from the wing vein after 7 weeks of treatment, using 3 ml syringes. Blood was divided into two parts; the first was mixed with EDTA, and used to estimate blood picture, whereas the second part was allowed for coagulation (26), centrifuged, and serum was collected to fresh tubes to be used for biochemical parameters.

Then, Bodyweight was recorded individually, and animals were sacrificed by cutting the jugular vein, and tissue samples were taken including, liver, kidneys, pancreas, heart, ovary and testes, dried on a filter paper, and weights of tissues were recorded relative to the total body weight (6). All specimens were frozen at -20 C.

## Laboratory tests

Blood picture was estimated, including; RBC, WBC, PCV, HB, MCV, MCH, MCHC according to Campbell (10). Lipid profile estimated in serum using photometric kits (Biolabs). The optical density for each was read using a spectrophotometer (Lovibona). Hormones were estimated using the ELISA technique by specific kits for each (Biosource).

## Statistic

Data were subjected to one-way analysis of variance. Significance was determined using Duncan Multiple tests, and considered at  $p \le 0.05$  (9). All statistical analyzes with the SPSS 10.00 pack.

## **RESULTS AND DISCUSSION**

The treatment with yeast and/or folic acid yielded significant effects on the blood picture of males quail (Table 1). Both groups treated with yeast showed a significant decrease in Hb compared to control, however, the most significant reduction was in the folic acidtreated group. Treatment with yeast caused an elevation in the PCV value, which started to decrease in both groups treated with folic acid. Treatment with yeast showed no effect on the RBC count, whereas a reduction was observed in the folic acid-treated groups. WBC count has elevated in the yeast-treated group but started to decrease in other groups where the yeast and the folic acid group showed the least significant value. Regarding both MCH and MCHC values, treatment with yeast caused a significant decrease compared to the control, but folic acid with yeast caused an elevation in MCH and a drop in MCHC compared to the control group. Finally, the treatments caused an increase in MCV value compared to the control, consider in the least value was in the yeast group.

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Table 1. Male Blood Profile in	quails treated with saccharomyce	es cerevisiae and/or folic acid

Blood	Hb	PCV	RBC	WBC	МСН	MCHC	MCV
				$*10^{3}$	-		
profile	g/dl	%	*10 <sup>6</sup>		pg	g/dl	fl
Groups			Cell/ml	Cell/ml			
Control	21.8	42.6	4.49	27.48	47.58	51.27	92.97
	±	±	±	±	±	±	±
	0.06	0.65	0.02	0.42	0.26	0.72	1.54
	а	с	а	с	b	a	с
Yeast	20.20	48.8	4.76	38.53	43.48	42.08	103.55
	±	±	±	±	±	±	±
	0.37	0.53	0.08	0.35	0.53	0.42	2.06
	b	а	а	а	с	с	b
Folic acid	17.64	37.3	3.43	33.5	48.36	46.86	111.38
	±	±	±	±	±	±	±
	0.44	0.53	0.12	0.86	0.44	0.63	1.6
	с	b	с	b	b	b	а
Yeast	20.66	44.4	3.99	21.0	50.89	46.54	109.45
+ Folic	±	±	±	±	±	±	±
acid	0.3	0.68	0.11	0.3	0.85	0.29	2.37
	b	b	b	d	а	b	а
s are express	ed as mean	±SE. n= 10		indicato	rs showed	a signification	nt decrea

- Different letters in each column refer to significance ( $P \le 0.05$ )

Similar to data shown in table 1, the treatments caused changes in the blood picture of females quail (Table 2). Both Hb and PCV values have decreased in all treatments compared to the control. Folic acid treatment decreased the RBC count amongst other groups, whereas the treatment with yeast elevated the WBC count amongst other groups. The blood picture indicators showed a significant decrease only when quails were treated with yeast, with no effects of other treatments. Table 3 shows the body weight of female quails exposed to yeast and/or folic acid. Bodyweight decreased after treatment with yeast and folic acid separately but started to elevated of quails when treated with both. Similarly, body weight changes in males decreased in the same manner seen in females (Table 4).

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Table 2. Female Blood Profile in c	Jualis treated with saccharomyces	<i>cerevisiae</i> and/or lolic acid

Blood profile	Hb	PCV	RBC	WBC	MCH	MCHC	MCV
	g/dl	%	*10 <sup>6</sup>	*10 <sup>3</sup>	pg	g/dl	fl
			Cell/ml	Cell/ml			
Groups							
Control	20.93	43.3	4.91	26.92	43.27	48.51	88.83
	±	±	±	±	±	±	±
	0.35	0.65	0.17	0.65	2.06	1.41	2.23
	а	a	ab	bc	а	а	а
Yeast	17.93	40.5	4.97	43.45	36.11	44.38	81.47
	±	±	±	±	±	±	±
	0.13	0.68	0.09	0.18	0.56	0.78	1.14
	с	b	а	a	b	b	b
Folic acid	17.1	36.0	3.94	28.62	43.45	47.68	91.24
	±	±	±	±	±	±	±
	0.08	0.78	0.08	1.19	0.7	0.95	1.15
	с	с	c	b	а	a	а
Yeast	19.4	41.2	4.61	25.16	42.06	47.02	89.27
+ Folic acid	±	±	±	±	±	±	±
	0.56	0.57	0.02	0.12	1.38	0.85	1.6
	b	b	b	с	a	ab	a

- Values are expressed as mean ±SE. n= 10

- Different letters in each column refer to significance ( $P \le 0.05$ )

The weights of the ovary elevate only in the yeast group compared to others, whereas the weight of the kidney decreased only in the group received both yeast and folic acid. The weight of the heart of females decreased only in the yeast group, while treatments with folic acid caused an elevation in the pancreas weight. Noteworthy, no significant change has been observed in liver weigh in females (Table 3).

Table 3. Female weight in quails treated with saccharomyces cerevisiae and/or folic acid

Weights	BW	Ovary	Liver	Kidney	Heart	Pancreas
0	gram	g/100g	g/100g	g/100g	g/100g	g/100g
Groups		B.W.	B.W.	B.W.	B.W.	B.W.
Control	233.5	3.79	2.97	0.67	0.82	0.23
	±	±	±	±	±	±
	4.0	0.28	0.19	0.02	0.03	0.01
	а	b	а	а	а	b
Yeast	204.5	5.08	2.99	0.66	0.71	0.23
	±	±	±	±	±	±
	3.2	0.27	0.06	0.02	0.05	0.01
	с	а	а	а	b	b
Folic acid	199.3	3.34	3.03	0.61	0.86	0.3
	±	±	±	±	±	±
	0.53	0.19	0.06	0.03	0.01	0.01
	с	b	а	а	а	a
Yeast	219.0	3.32	2.99	0.51	0.81	0.32
+ Folic acid	±	±	±	±	±	±
	5.4	0.2	0.09	0.02	0.05	0.02
1	b	b	а	b	а	а

- Values are expressed as mean  $\pm$ SE. n= 10 -Different letters in each column refer to significance (P  $\leq 0.05$ 

All treatments caused an elevation in the weights of testes in male quails compared to the control. Liver weight has also elevated as a result of treating male quails with yeast and/or

folic acid. The only elevation observed in the kidney weight was after the treatment with folic acid, whereas all the three treatments yielded a significant elevation in both heart and pancreas weights compared to the control. (Table 4).

Weights	BW	<b>R.Testis</b>	L. Testis	Liver	Kidney	Heart	Pancreas
	gram	g/100g	g/100g	g/100g	g/100g	g/100g	g/100g
Groups		B.W.	B.W.	B.W.	B.W.	B.W.	B.W.
Control	219.5	1.38	1.51	1.35	0.43	0.8	0.14
	±	±	±	±	±	±	±
	11.4	0.1	0.08	0.06	0.02	0.02	0.01
	а	с	b	с	bc	b	с
Yeast	154.5	1.66	2.1	1.65	0.38	0.99	0.21
	±	±	±	±	±	±	±
	1.1	0.07	0.1	0.02	0.02	0.02	0.01
	с	ab	а	b	с	а	а
Folic acid	168.5	1.74	1.94	1.83	0.53	0.93	0.17
	±	±	±	±	±	±	±
	1.2	0.05	0.02	0.03	0.03	0.04	0.01
	с	а	а	а	a	а	b
Yeast	187.0	1.48	1.68	1.65	0.46	0.96	0.21
+ Folic	±	±	±	±	±	±	±
acid	4.99	0.08	0.03	0.02	0.01	0.01	0.01
	b	bc	b	b	b	а	а

Table 4 Male Weights in quails trooted with accelera •• •

- Values are expressed as mean  $\pm$ SE. n= 10

- Different letters in each column refer to significance ( $P \le 0.05$ )

Table 5 shows the biochemical parameters observed by treating male quails with yeast and/or folic acid. TG decreased in all treated groups compared to there control, however, the most significant decrease has been shown in the group received folic acid. TC decreased similarly in all treatments compared to the control. In general, the treatment with yeast did not affect protein metabolism indicators including TP and Alb, whereas the treatment with both yeast and folic acid led to decrease

proteins concentration in the blood, and the treatment with folic acid increased Alb concentration amongst the treated groups. Regarding the hormones, the treatment with folic acid caused an increase in cortisol compared to the control, which shows no significance with other treated groups. The concentration of testosterone hormone has shown an elevation in all treatments, with the highest value observed in the yeast group, folic acid, and finally folic acid and yeast group respectively.

Parameters	TG	TC	Albumin	ТР	Cortisol	Testosterone
Groups	mg/dl	mg/dl	g/dl	g/dl	mg/dl	ng/dl
Control	189.7	282.9	1.18	3.6	2.71	3.21
	±	±	±	±	±	±
	2.7	1.35	0.01	0.03	0.04	0.05
	а	а	b	а	b	d
Yeast	110.1	169.7	1.23	2.78	2.76	7.55
	±	±	±	±	±	±
	3.5	6.28	0.03	0.16	0.03	0.04
	с	b	b	b	ab	а
Folic acid	80.0	164.1	1.35	3.58	2.82	5.75
	±	±	±	±	±	±
	1.13	8.6	0.05	0.07	0.01	0.04
	d	b	а	а	а	b
Y+F	165.3	181.3	1.01	2.93	2.77	3.86
	±	±	±	±	±	±
	2.2	8.3	0.02	0.08	0.03	0.14
	b	b	с	b	b	с

Table 5 . Some Biochemicals and Hormonal aspects in Male quails treated with
saccharomyces cerevisiae and/or folic acid

- Values are expressed as mean  $\pm$ SE. n= 10.

- Different letters in each column refer to significance ( $P \le 0.05$ )

Regarding the metabolic and hormones values in females, table 6 shows that the only decrease in TG is a result of treating quails with folic acid. TC elevated as a result of all treatments, giving the highest value in yeasttreated groups, then the folic acid group, which is also significantly higher than the control. The treatment with yeast caused an elevation in TP compared to control, however, folic acid has a role in reducing this value. Yeast and folic acid separately have a role in elevating Alb over the control, but can reduce the value when given together. No significant change was observed in such cortisol, LH, and FSH concentrations in all treatments.

Table 6 . Some Biochemicals and Hormonal aspects in Female quails treated with
saccharomyces cerevisiae and/or folic acid

Parameters	LH	FSH	Cortisol	ТР	Albumin	ТС	TG
Groups	IU/L	IU/L	mg/dl	g/dl	g/dl	mg/dl	mg/dl
Control	0.86	0.86	2.86	4.14	1.5	119	498
	±	±	±	±	±	±	±
	0.013	0.013	0.014	0.04	0.017	1.17	0.51
	a	а	a	с	с	d	а
Yeast	0.87	0.87	2.86	6.26	2.54	288.4	496.8
	±	±	±	±	±	±	±
	0.011	0.01	0.014	0.1	0.02	1.97	1.5
	a	а	a	a	b	а	а
Folic acid	0.86	0.87	2.87	4.36	2.74	168.6	492.9
	±	±	±	±	±	±	±
	0.012	0.011	0.01	0.09	0.02	2.15	1.96
	a	а	а	с	а	с	b
Y+F	0.89	0.88	2.87	5.49	1.29	191.3	498.2
	±	±	±	±	±	±	±
	0.004	0.005	0.014	0.04	0.02	1.45	0.55
	a	a	a	b	d	b	а

- Values are expressed as mean ±SE. n= 10

-Different letters in each column refer to significance  $(P \le 0.05)$ 

This study investigates the role of yeast (saccharomyces cerevisiae) and/or folic acid in improving physiological and biochemical characteristics in Quails. We found interesting results of both materials used as well as a synergistic effect of using them together in terms of enhancing desired characteristics in quails. These characteristics might be used as indicators of productivity and reproduction in poultries and could be applied in other if applicable. However, animals, some unexpected results have also been found.We deliberately presented the results observed from males separated from those from females. This is to avoid any interference of the body physiology of such with the parameters. And a consequent coincidence. Intriguingly, the treatment with yeast and/or folic acid resulted in a decrease in the blood profile parameters, PCV, Hb, and RBC count, in both males and females (Table1&2). This result does not agree with (18) findings regarding the treatment with yeast. On the other hand, the elevation of WBC count resulted from the treatment with yeast or folic acid separately is in agreement with (28). Unexpectedly, the treatment with folic acid has also shown a decrease in blood profile. The current findings might be attributed to an

early stage of anemia as a result of egg production, which starts earlier in quails compared to hens. It is also suggested that the treatments with yeast and/or folic acid may have an inhibitory effect on the expression of either IL-6 or  $\alpha$ -TNF or both in quails, which explains these findings (3, 4). Overall, our results in this context suggest different physiological mechanisms in quails to maintain their body homeostasis. As shown in tables 5, the treatment with yeast and/or folic acid caused a significant decrease in both TC and TG in males, but not in females. Literature refers to a hypolipidemic effect on yeast when given to animals (12,21,30). This effect might be attributed to the activation of enzymes, responsible liver for lipid metabolism, undertaken by saccharomyces administration cerevisiae (30).The administration of yeast did not affect the albumin level but managed to reduce it when given with folic acid. Furthermore, yeasted reduced the total protein level with or without folic acid in male quails. On the other hand, all treatments caused an elevation in both albumin and total protein in females. This is partially in agreement with (5). However, it was reported that dietary yeast improves protein metabolism as a result of improving liver enzymes as well as anti-protease enzyme activity (29). The effects of folic acid administration observed in this study, on the levels of both lipids and proteins are explained by the impact of folic acid to decrease homocysteine concentration, and herby apolipoproteins A and B, which explains the variations of folic acid action between males and females (5). The only significant effect of males cortisol level has been observed in the group treated with folic acid. This is in agreement with (19), who found a positive correlation between cortisol and folic acid levels. The molecular mechanism for these effects is still unknown, however. some assumptions have been proposed based on some laboratory findings such as the regulatory effect of folic acid on plasma homocysteine and the reduction of membrane docosahexaenoic acid (DHA) (19). Worth noting, these effects have not been seen females. which suggests different in mechanisms controlled by different body physiology. We observed an enhanced level of testosterone in males as a result of all treatments. Whereas no effect has been detected with female hormones. The most significant increase in testosterone level was in the group treated with yeast, and the last one was in the group treated with both yeast and folic acid. Literature did not refer to the effects of yeast administration on steroid sex hormone, however, the effect could result from the activation of one or more enzymes catalyzing the synthesis pathways of these hormones. In this context, more investigations are needs. On the other hand, folic acid has been found to have positive effects on testicular function (32). This effect is presumably attributed to the ability of folic acid for scavenging reactive oxygen species Surprisingly, supplementation (ROS) (11). with yeast and/or folic acid reduced the bodyweight of treated birds (Table 3). This result is not in agreement with Ahiwe et al. (2019) and Ryu et al. (1995) (2, 31). Indeed, no scientific explanation for this result thus far, however, it may be due to the high energy demand during the egg production period, which further depletes the body mass in the treated groups vs control. In contrast to the body weight, testes weight increased in both yeast and folic acid groups, which matches the results of (8, 32). This confirms our results regarding testosterone level (Table 5), which suggests that our treatments improve the testes' function. A variation has been detected in this study in terms of organ weights (liver, kidney and heart) between males and females. Treatments caused an increase in organ weights in males, but not in females (Table 3), which may be as a consequence of physiological differences between males and females. These results confirm the findings of (13, 27). Mutavdzin et al, 2019 (27), who found that folic acid treatments improve the metabolic status by means of improving liver and kidney functions, and thereby enhancing body homeostasis. Furthermore, treatment with yeast has been found to participate detoxifying effects in kidney and liver, hence it scavenges a variety of toxins and free radicals, therefore an improvement of liver and kidney functions is expected (23). Overall, the results of this study found interesting effects of yeast or folic acid on the studied parameters in quails, however, the combination of both of them vielded less or non-effects, which suggests a kind of interaction between them, interfering the desired effects.

## Conclusion

Unexpectedly, treating quails with saccharomyces cerevisiae and/or folic acid did not reveal desired effects on different physiological and biochemical parameters in normal males and females. However, treating males resulted in an enhancement of regulation testosterone and of lipids metabolism.

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## **CONFLICT OF INTEREST**

There is no conflict of interest.

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