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Response of Broilers Performance to Dietary Betaine and Folic Acid at Different Methionine Levels

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Abstract: Two experiments were designed to estimate the effect of methionine levels (0.33 and 0.45%) with betaine and folic acid on broiler performance. A total of 648 unsexed one week old Arbor Acres broiler chicks was randomly divided into two experiments according to dietary methionine level. Each experiment divided into nine treatment groups of 12 birds each with three replicates. The experimental diets were formulated to cover the nutrients requirements for broilers and were supplemented with betaine at 0.5, 0.75 or 1.0 gm kg⁻¹. Folic acid was added at 0.5, 0.75 or 1.0 mg kg⁻¹ for each betaine level. Results can be summarized as follows: Live body weight gain and feed conversion efficiency were significantly increased with increasing folic acid addition and increased with increasing betaine levels up to 0.75 gm kg⁻¹ diet. Productive performance was significantly improved by increasing different levels of betaine and folic acid. The OM, CP, EE, CF and NFE digestion coefficients were significantly ($p>0.5$) increased with increasing betaine or folic acid levels in the diets. The improvement of chick performance due to added betaine was depressed when chicks received diets containing recommended methionine, whereas, chicks performance improved by increasing folic acid level. Folic acid had significant effect on dressing %, the highest level received the highest dressing % recorded, while no significant effects were noticed in digestion coefficients of nutrients. Blood plasma AST and ALT decreased with increasing dietary methionine level. The highest economic efficiency was listed when diet contained the highest levels of betaine and folic acid.

Key words: Methionine, betaine, folic acid, performance, broilers

Introduction

Numerous poultry trials have been previously conducted to establish the dependency, functionality and requirement for methionine supplementation in animal diets. Addition of methionine to the birds diet has been correlated with the tendency to have less total body fat (Rostagno *et al.*, 1995). Methionine has been supplemented to overcome growth depression even when caused by dietary tannic acid and mild arginine toxicity, with methyl donors in general (Kim *et al.*, 2006). The liver is an important site for methionine metabolism and methionine is an amino acid of critical importance in commercial poultry diets, because it is typically the first limiting amino acid. Methionine, betaine and choline are all sources of labile methyl groups play an important role in methylation reactions and the methyl group metabolism of these three compounds is interrelated as illustrated by Pillai *et al.* (2006a). Betaine is a tertiary amine formed by the oxidation of choline (Wang *et al.*, 2004) and implicated in methionine sparing, osmo-protective and fat distribution (Tur Ker *et al.*, 2004). Young broiler chickens fed diets containing practical ingredients may respond (improved growth and feed utilization) to supplements of folic acid if there are no supplements of the labile methyl donating compounds methionine or choline. Folic acid values in ingredient composition tables may be lower than they should be in

some cases, but higher in others. For ingredients like soybean meal, processing methods changed from an expeller process to hexane extraction. Because hexane extraction results in the removal of more folic acid from modern soybean meal, soybean meal may contain less folic acid than is indicated in ingredient composition table. Supplemental choline replaced methionine efficiently enough to be able to decrease the supplemental L. methionine level in the diet, but this methylation reaction appeared to be inefficient without adequate dietary supplemental folic acid (Pesti *et al.*, 1991).

Frits (2005) illustrated the pathways of betaine, folic acid and methionine (Fig. 1).

These studies were designed to investigate the influence of methionine levels supplemented with different levels of betaine and folic acid in broiler diets on performance, digestibility, carcass traits and blood plasma parameters.

Materials and Methods

A total of 648 unsexed one-week-old Arbor Acres broiler chicks were randomly and equally divided into two experiments. The first experiment (Exp I) was given diets containing low level of DL-methionine (0.33%), while, the second experiment (Exp II) was given diets containing normal level of DL-methionine (0.45%). Each

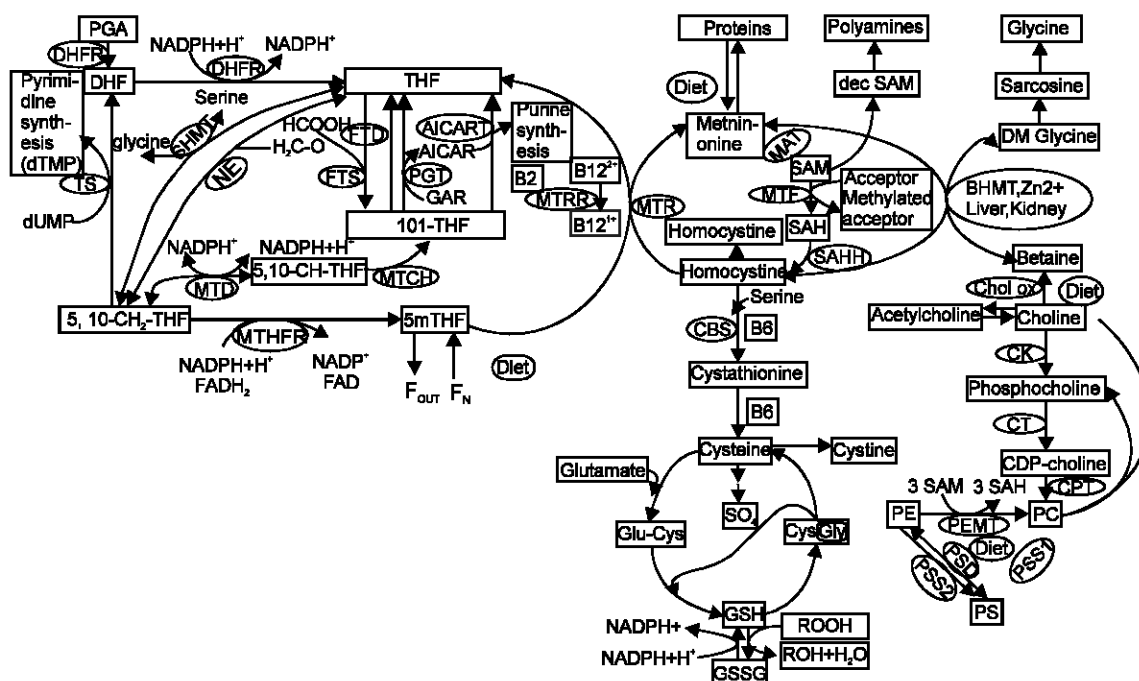


Fig. 1: The pathway of betaine, folic acid and methionine

Where: 10f-THF: 10-formyltetrahydrofolate; 5,10-CH₂-THF: 5,10-methylenetetrahydrofolate; 5,10-CH₂-THF: 5,10-methylenetetrahydrofolate; 5mTHF: 5-methyltetrahydrofolate; AICAR: aminoimidazolecarboxamide ribotide; AICART: aminoimidazolecarboxamide ribotide transformylase; BHMT: betaine homocysteine methyl transferase; CBS: cystathionine β-synthase; CDP: cytidine diphosphate; Chol ox: choline oxidase; CK: choline kinase; CPT, CDP-choline:1,2-diacylglycerol cholinephosphotransferase; CT, CTP-phosphocholine cytidylyltransferase; Cys: cysteine; Cys-Gly, cysteinylglycine; decSAM: decarboxylated S-adenosyl methionine; DHF: dihydrofolate; DHFR: dihydrofolate reductase; DMGlycine: dimethylglycine; dTMP: thymidine monophosphate; dUMP: 2′deoxyuridine monophosphate; FAD (H₂): oxidized (reduced) flavin adenine dinucleotide (vitamin B₂); Fin and Fout: the rates at which 5 mTHF enters and leaves the cell, respectively; FTD, 10-formyltetrahydrofolate dehydrogenase; FTS, 10-formyltetrahydrofolate synthase; GAR: glycinamide ribotide; Glu: glutamine; Glu-Cys: glutamylcysteine; Gly: glycine; GSH: reduced glutathione (Glu-Cys-Gly); GSSG, oxidized glutathione; MAT, methionineadenosyltransferase; MTR, methionine synthase; MTHFR: 5,10-methylenetetrahydrofolate cyclohydrolase; MTD: 5,10-methylenetetrahydrofolate dehydrogenase; MTF, methyltransferases (including DNA methyltransferases); MTHFR, 5,10-methylenetetrahydrofolate reductase; MTRR, methionine synthase reductase; NADP(H), oxidized (reduced) nicotinamide adenine dinucleotide phosphate; NE, nonenzymatic interconversion of THF and 5,10-CH₂-THF; PC: phosphatidylcholine; PE, phosphatidylethanolamine; PEMT: phosphatidylethanolamine N-methyl transferase; PGA, pteroyl-l-glutamic acid (folic acid); PGT, phosphoribosyl glycinamidetransformylase; PS: phosphatidylserine; PSD: phosphatidylserine decarboxylase; PSS1 and 2: phosphatidylserine synthase; ROOH: peroxide; SAH, S-adenosylhomocysteine; SAHH: S-adenosylhomocysteine hydrolase

experiment divided into nine treatment groups (3 replicates per group, each of 12 birds) (Table 1). All chicks were brooded floor pens and kept in temperature controlled and similar management conditions. Lighting program was provided about 23 hours daily. Feed and water were offered ad-libitum during the experimental period which lasted for seven weeks. All chicks were fed commercial diet during the first week of age before receiving the experimental diets. From 1 to 4 week old. The chicks fed starter diets with 3100 Kcal ME Kg⁻¹ and 23 and CP and finisher diets with 3200 Kcal ME Kg⁻¹ and 20 and CP during finishing period (from 5 to 7 week

old). All diets were supplemented with different levels of betaine (as betafene which extracts from sugar beet molasses) and folic acid. Each treatment group of either experiments Exp. I 0.33% DL-methionine and Exp.II 0.45% methionine received one of the following diets:

The experimental diets were formulated to satisfy the nutrients needed as recommended by the NRC (1994), (Table 2). At the end of the experiment, 54 birds (3/treatment) were randomly chosen to carry out a digestion trial to determine nutrients digestibility of each experimental diet. These birds were fed on the tested diets for 5 days collection period. Chemical analyses of

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Table 1: Experimental design

T	Betaine	folic acid	T	Betaine	folic acid
T1	0.50 g kg ⁻¹ diet	0.50 mg kg ⁻¹ diet	T6	0.75 g kg ⁻¹ diet	1.00 mg kg ⁻¹ diet
T2	0.50 g kg ⁻¹ diet	0.75 mg kg ⁻¹ diet	T7	1.00 g kg ⁻¹ diet	0.50 mg kg ⁻¹ diet
T3	0.50 g kg ⁻¹ diet	1.00 mg kg ⁻¹ diet	T8	1.00 g kg ⁻¹ diet	0.75 mg kg ⁻¹ diet
T4	0.75 g kg ⁻¹ diet	0.50 mg kg ⁻¹ diet	T9	1.00 g kg ⁻¹ diet	1.00 mg kg ⁻¹ diet
T5	0.75 g kg ⁻¹ diet	0.75 mg kg ⁻¹ diet			

Table 2: Composition and calculated analysis of the experimental diets

Ingredient%	Starter ration		Finisher ration	
	0.33% methionine	0.45% methionine	0.33% methionine	0.45% methionine
Yellow com	48.60	48.50	55.50	55.40
Soybean meal (44%)	40.00	40.00	30.60	30.60
Corn glutine meal (62%)	2.00	2.00	3.90	3.90
Vegetable oil	5.90	5.90	6.60	6.60
Di-calcium phosphate	1.70	1.70	1.80	1.80
Limestone	1.20	1.18	1.00	0.98
Sodium chloride	0.30	0.30	0.30	0.30
Vit. Min. Pre mix.*	0.30	0.30	0.30	0.30
DL-methionine	-	0.12	-	0.12
Calculated analysis **				
Crude protein%	22.96	22.96	20.06	20.06
ME Kcal/kg	3102	3102	3230	3230
Lysine%	1.24	1.24	1.10	1.10
Methionine%	0.33	0.45	0.33	0.45
Cystine%	0.37	0.49	0.34	0.46
Calcium%	0.96	0.96	0.89	0.88
Available-p%	0.46	0.45	0.45	0.45

*Vitamins and minerals premix were free from folic acid: each kg contains vit A 12000 lu, vit.D3 3000 lu, vit. E 12 mg, vit. K 1mg, vit B12 0.02mg, vit B1 1mg, vit B2 4mg, vit B6 5mg, Nicotinic acid 20 mg, Biotin 0.05 mg, Choline chloride 0.16 mg, copper 3 mg, iron 30 mg, manganese 40 mg, zinc 45 mg and selenium 3 mg, **According to N.R.C. (1994)

tested diets and dried excreta were determined according to A.O.A.C. (1990). Faecal nitrogen was determined following the procedure outlined by Jacobsen *et al.* (1960).

At the end of digestion trials the birds deprived from feed for 12 hours, then weighed and slaughtered to estimate some carcass characteristics. During slaughtering, blood samples were collected from the wing vein in heparinized tubes and centrifuged (3000 rpm/15 minutes). The plasma was obtained immediately and stored at -20°C until analysis for plasma Total Protein (TP), Albumin (ALB), Cholesterol (ChOL), Total Lipids (T.LIPS), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using commercial kits purchased from Bio-Merieux (Morcyl Etiols Charbon Mierels Rains/France).

The economic efficiency for meat production was calculated based on the prices of MFA sources and feed ingredients prevailing 2005.

Statistical analysis: Data were statistically analyzed using the general linear model for analysis of variance of SAS (SAS, 1990). Test of significance for the differences between means of different levels within each classification was done by Duncan's multiple range test (Duncan, 1955).

Results and Discussion

Broiler performance

Experiment I: The live body weight, gain in weight, feed consumption, conversion efficiency and dressing percentages are summarized in Table 3. Body weight gain and the efficiency of feed conversion were significantly increased linearly ($p < 0.05$) with increasing folic acid addition and increased ($p < 0.05$) with increasing betaine levels up to 0.75 gm kg⁻¹ diet, while no significant differences were noticed between treatments in feed intake and dressing percentage due to the levels of betaine or folic acid, except at 0.50 or 1.00 mg folic acid/kg diet, dressing % being significantly more. Moreover, significant interactions ($p < 0.05$) between betaine and folic acid, mainly T6 and T9 recorded the highest values in body weight and body weight gain compared with T1 which recorded the lowest value, while T1 consumed much feed with the last conversion efficiency. (2.11 ± 0.04) compared to T9 which recorded the highest efficiency (1.80 ± 0.03), this interpret that T9 surpassed the other treatments.

The differences in dressing percentages were not significant due to betaine levels However, there was a significant effect ($p < 0.05$) in dressing percentage due to either folic acid or interaction between betaine and folic acid, these effects were in irregular trend and ranged

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Table 3: Effect of different levels of betaine and folic acid on broilers performance at low level of methionine, 0.33% (Exp. I)

Item	I. Body Weight	f. Body Weight	Body Weight Gain (gm)	Feed Intake	Feed Conversion	Dressing -----%
Betaine (B) effects						
B1 (0.50 gm/kg)	94.71±0.29	1659.51 ^b ±12.40	1564.79 ^b ±12.53	3152.1±42.71	2.02 ^b ±0.03	66.58±0.70
B2 (0.75 gm/kg)	94.71±0.12	1722.31 ^a ±19.72	1627.60 ^a ±19.69	3048.6±34.59	1.87 ^b ±0.02	68.71±1.03
B3 (1.00 gm/kg)	94.86±0.13	1747.08 ^a ±17.81	1652.22 ^a ±17.83	3046.4±27.95	1.84 ^b ±0.02	67.00±0.55
Folic acid (F) effects						
F1 (0.50 mg/kg)	94.83±0.23	1661.30 ^c ±11.94	1566.46 ^c ±12.04	3086.3±52.82	1.97 ^a ±0.04	68.03 ^{ab} ±1.03
F2 (0.75 mg/kg)	94.69±0.20	1712.82 ^b ±15.84	1618.13 ^b ±15.83	3066.0±35.29	1.90 ^b ±0.02	65.97 ^b ±0.65
F3 (1.00 mg/kg)	94.76±0.16	1754.79 ^a ±20.68	1660.03 ^a ±20.64	3094.7±25.00	1.87 ^b ±0.03	68.29 ^a ±0.52
B x F effects						
(T1) B1 x F1	94.97±0.61	1630.41 ^d ±19.23	1535.44 ^d ±19.83	3242.5 ^b ±63.47	2.11 ^a ±0.04	66.51 ^{ab} ±1.14
(T2) B1 x F2	94.53±0.58	1668.47 ^{cd} ±23.35	1573.95 ^{cd} ±23.71	3088.0 ^{ab} ±98.23	1.96 ^b ±0.04	65.18 ^b ±1.42
(T3) B1 x F3	94.64±0.49	1679.64 ^{cd} ±61.21	1585.00 ^{cd} ±15.85	3125.6 ^{ab} ±41.55	1.97 ^b ±0.04	68.05 ^{ab} ±1.02
(T4) B2 x F1	94.75±0.34	1664.86 ^{cd} ±16.47	1570.11 ^{cd} ±16.19	3002.2 ^b ±65.96	1.91 ^{bc} ±0.03	70.61 ^a ±2.42
(T5) B2 x F2	94.53±0.19	1715.97 ^{bc} ±16.55	1621.44 ^{bc} ±16.68	3056.4 ^{ab} ±65.35	1.88 ^{bc} ±0.02	66.29 ^{ab} ±1.01
(T6) B2 x F3	94.86±0.03	1768.11 ^a ±20.39	1691.25 ^a ±20.38	3087.1 ^{ab} ±62.39	1.83 ^{cd} ±0.02	69.22 ^{ab} ±0.95
(T7) B3 x F1	94.78±0.37	1688.61 ^{cd} ±14.56	1593.84 ^{cd} ±14.84	3014.3 ^b ±82.29	1.89 ^{bc} ±0.03	66.99 ^{ab} ±0.80
(T8) B3 x F2	95.03±0.07	1754.03 ^{ab} ±18.81	1659.00 ^{ab} ±18.74	3053.6 ^{ab} ±25.60	1.84 ^{cd} ±0.01	66.43 ^{ab} ±1.41
(T9) B3 x F3	94.78±0.24	1798.61 ^a ±13.33	1703.83 ^a ±13.50	3071.3 ^{ab} ±33.11	1.80 ^d ±0.03	67.58 ^{ab} ±0.80

a,b,c,d: Means in the same column with different letters are significantly different (p<0.05)

between 65.18±1.42% to 70.61±2.42% for T2 and T4, respectively. These results agreed with the findings of Turker *et al.* (2004) who reported that betaine supplementation to 0.32 or 0.37 and methionine containing-diets significantly improved growth and feed conversion. Zhan *et al.* (2006) found that methionine and betaine supplementation significantly improved weight gain and feed conversion. Esteve-Garcia and Mach (2000) reported that betaine may not substitute methionine and chicks fed low methionine diets containing 500 g kg⁻¹ isolated soybean protein, responded positively to either methionine or choline or betaine. Chavez and Kratzer (1974) studied the dermatitis in turkeys fed soybean and reported that homocystine or betaine enhanced growth, but could not alleviate a food pad dermatitis caused by methionine deficiency, indicating that betaine could not replace methionine in all of its functions. However, Wang *et al.* (2004) showed that betaine significantly improved growth and feed conversion ratio of ducks fed methionine adequate diets. The present dressing % agreed with those reported by Kermanshahi (2001) who found that the carcass characteristics were not affected by betaine replacement.

The improvement in growth performance due to betaine may be due to its effect as methyl donor as already methionine and its diverse physiological properties that could improve gut environment and thus enhance the ability of the chicks to withstand coccidial infection (Remus *et al.*, 2004). The performance due to dietary folic acid was supported by Kyeong Seon Ryu *et al.* (1995) who observed that growth and feed conversion responses to folic acid supplementation were both curvilinear, reaching maximum at 1.45 mg kg⁻¹ diet and then declining. These values corresponded to 1.3 and 1.2 mg kg⁻¹ total folic acid based on the improved

method of folic acid measurement that was used. Moreover, Hebert *et al.* (2004) found no significant difference (p>0.05) in feed consumption or feed conversion ratio due to folic acid supplementation up to 4 mg kg⁻¹ to the diet. El-Husseiny *et al.* (2005) reported that body weight gain and feed conversion ratio increased gradually with increasing supplemental folic acid but feed consumption was not affected.

Experiment II: The live body weight, gain in weight, feed consumption and conversion and dressing % are listed in Table 4, The differences in body weight, weight gain, feed intake, feed conversion and dressing % due to different levels of betaine were not significant (p>0.05). In addition, the effect of folic acid on body weight and weight gain was significant (p<0.05) and increased with increasing of folic acid level in the diet. A significant effect (p<0.05) on the body weight, body weight gain and dressing % due to betaine×folic acid interaction, T9 recorded the highest values (1823.03±14.30 and 1728.16±14.10) followed by T6 (1798.73±19.63 and 1703.58±19.61) compared to T1 which recorded the lowest values (1714.58±20.65 and 1619.67±20.79) for body weight and body weight gain, respectively. Dressing percentage values ranged between (66.86±1.10 and 63.75±0.40) for T1 and T4, respectively. Pillai *et al.* (2006a) observed that the main effect of choline and betaine addition on the basal diet containing deficient level of methionine (0.28%) was significant (p<0.05) and the weight gain, feed intake and efficiency was increased. The same response was reported by Pillai *et al.* (2006b) in birds fed diets deficient in methionine and cystine with surfeit choline or betaine supplementation. Furthermore, these notes are similar to those of Emmert *et al.* (1996) who noted a small increase in weight gain and feed efficiency of chicks fed

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Table 4: Effect of different levels of betaine and folic acid on broilers performance at normal level of methionine, 0.45% (Exp. II)

Item	l. Body Weight	f. Body Weight	Body Weight Gain	Feed Intake	Feed Conversion	Dressing
	(gm)					%
Betaine (B) effects						
B1 (0.50 gm/kg)	94.62±0.19	1770.46±16.32	1675.84±16.41	3133.7±30.46	1.87±0.02	65.18±0.67
B2 (0.75 gm/kg)	94.86±0.24	1772.37±12.42	1678.51±12.29	3123.7±55.24	1.86±0.02	64.28±0.37
B3 (1.00 gm/kg)	94.76±0.20	1791.75±11.09	1696.99±11.11	3108.4±27.79	1.83±0.01	64.58±0.30
Folic acid (F) effects						
F1 (0.50 mg/kg)	94.69±0.23	1740.79 ^b ±12.51	1646.09 ^b ±12.57	3086.7±37.55	1.87±0.02	65.16±0.58
F2 (0.75 mg/kg)	94.73±0.23	1788.43 ^a ±6.88	1693.69 ^a ±6.89	3140.9±26.52	1.85±0.02	64.72±0.27
F3 (1.00 mg/kg)	94.82±0.17	1806.37 ^a ±9.26	1711.55 ^a ±9.23	3143.2±48.72	1.84±0.03	64.16±0.51
B x F effects						
(T1) B1 x F1	94.92±0.46	1714.58 ^c ±20.65	1619.67 ^c ±20.79	3116.6±86.24	1.92 ^a ±0.03	66.86 ^a ±1.10
(T2) B1 x F2	94.50±0.35	1799.44 ^a ±13.97	1704.94 ^a ±14.05	3188.8±27.13	1.87 ^{ab} ±0.03	64.29 ^a ±0.59
(T3) B1 x F3	94.45±0.12	1797.36 ^a ±15.17	1702.92 ^a ±15.26	3095.8±24.08	1.82 ^b ±0.03	64.38 ^{ab} ±1.33
(T4) B2 x F1	94.47±0.43	1740.14 ^{bc} ±17.30	1645.67 ^{bc} ±16.88	3037.1±20.50	1.85 ^{ab} ±0.02	63.75 ^b ±0.40
(T5) B2 x F2	94.97±0.53	1781.25 ^{ab} ±16.17	1686.28 ^{ab} ±16.09	3101.2±68.27	1.83 ^{ab} ±0.02	65.28 ^{ab} ±0.23
(T6) B2 x F3	95.15±0.36	1798.73 ^a ±19.63	1703.58 ^a ±19.61	3232.6±146.9	1.90 ^{ab} ±0.07	63.80 ^b ±0.82
(T7) B3 x F1	94.70±0.46	1767.64 ^{ab} ±21.18	1672.94 ^{ab} ±21.63	3091.3±86.11	1.85 ^{ab} ±0.03	64.87 ^{ab} ±0.44
(T8) B3 x F2	94.72±0.43	1784.58 ^{ab} ±4.20	1689.86 ^{ab} ±3.84	3123.7±32.82	1.85 ^{ab} ±0.02	64.60 ^{ab} ±0.45
(T9) B3 x F3	94.86±0.29	1823.03 ^a ±14.30	1728.16 ^a ±14.10	3101.1±17.46	1.79 ^b ±0.01	64.28 ^b ±0.70

a,b,c: Means in the same column with different letters are significantly different (p<0.05)

a marginally methionine deficient diet when dietary choline was added. However, a commonality among these reports is that the weight gain response to surfeit choline or betaine in diets deficient in SAA was not of the same magnitude as the response to methionine. Virtanen and Rumsey (1996) revealed a response to supplemental choline or betaine in diets marginally deficient methionine, so that a portion of the methionine requirement appeared to be spared. In this respect, the results of Zhan *et al.* (2006) suggested that betaine can spare methionine in its function as an essential amino acid and is as effective as methionine in improving performance and carcass quality of growing broilers if the diet is moderately deficient in methionine. Betaine supplementation had no significant effect on dressing %, these results disagreed with those reported by Waldroup and Fritts (2005) who found that betaine increased yielded significantly (p>0.05). They attributed the increase in carcass yielded of betaine supplemented group to its osmotic effects and increasing water retention.

The different responses of betaine in both two experiments may be due to that the addition of betaine to diets marginally deficient in sulfur amino acid resulted in a marginal increase in weight gain in broiler chicks, but the response was inconsistent. Addition of betaine to diets that were deficient in methionine and cysteine or methionine alone resulted in increased total remethylation and the magnitude of homocysteine remethylation associated with betaine addition was greater in diets deficient only in methionine. The proportion of remethylation through betaine-homocysteine-methyl transferase (BHMT) was reduced by addition of either DL-Methionine (DLM) or 2-hydroxy-4-methylthio butanoic acid (HMB) and was further lowered

by addition of betaine. Choline and betaine appeared to have a higher effect on Methionine Synthase (MS) dependent homocysteine (HCY) remethylation than BHMT-dependent remethylation and the overall contribution of MS to HCY remethylation was much greater than that expected under most conditions. With fairly similar remethylation responses to betaine but only isolated differences in the growth response (Pillai *et al.*, 2006a).

Concerning to folic acid supplementation, body weight and weight gain increased by increasing folic acid level in diets, this result was nearly similar to those reported by Featherston (1979) who found that a folic acid deficiency exerted a greater detrimental effect on chick growth. However, the result did not show any significant differences concerning feed intake or feed conversion due to folic acid level in diets. Similar results were reported by El-Husseiny *et al.* (2005) and Khalifah and Shahein (2006). In studies with rats and in vitro intestinal cell model systems, folic acid has been shown to be absorbed from the gut via a membrane-bound folate transport system (Said, 2004) that accepts both oxidized and reduced forms of the monoglutamated forms of folate. This process has been shown to be saturable in a number of model systems (Said, 2004) and as such represents a potential control point for plasma folate concentrations. However, the 5-methyltetrahydrofolat form is believed to be the primary circulating form of folate in the in the plasma and therefore the existence of other control points can not be dismissed. In the enterocyte, folic acid is reduced via a 2-step process involving the enzyme dihydrofolat reductase to yield tetrahydrofolat (Henderson, 1990). 5-methyltetrahydrofolat participates in a reaction involving the folate-dependent remethylation of the sulfur AA homocysteine to yield methionine.

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Table 5: Effect of different levels of betaine and folic acid on nutrients digestibility at low level of methionine,0.33% (Exp. I)

Item	Digestibilities %				
	OM	CP	EE	CF	NFE
Betaine (B) effects					
B1 (0.50 gm/kg)	76.29 ^c ±0.16	90.03 ^c ±0.18	73.62 ^b ±1.33	34.75±0.81	73.79 ^c ±0.28
B2 (0.75 gm/kg)	78.55 ^b ±0.87	90.93 ^b ±0.43	78.96 ^c ±0.70	35.56±2.29	76.16 ^b ±0.99
B3 (1.00 gm/kg)	80.38 ^a ±0.63	91.93 ^a ±0.45	80.41 ^a ±1.23	38.55±1.38	78.34 ^a ±0.87
Folic acid (F) effects					
F1 (0.50 mg/kg)	77.01 ^c ±0.52	90.52 ^b ±0.33	77.10 ^b ±1.12	33.64 ^b ±0.84	74.23 ^b ±0.65
F2 (0.75 mg/kg)	78.30 ^b ±0.77	90.47 ^b ±0.43	74.95 ^c ±1.90	36.47 ^{ab} ±1.70	76.63 ^a ±0.95
F3 (1.00 mg/kg)	79.90 ^a ±0.92	91.89 ^a ±0.44	80.94 ^a ±1.48	38.74 ^a ±1.88	77.43 ^a ±1.05
B x F effects					
(T1) B1 x F1	76.07 ^b ±0.30	89.91 ^c ±0.37	74.25 ^{bc} ±0.38	34.69 ^{ab} ±1.08	73.37 ^b ±0.45
(T2) B1 x F2	76.24 ^b ±0.21	89.75 ^c ±0.07	71.24 ^c ±3.93	35.05 ^{ab} ±2.37	74.26 ^b ±0.45
(T3) B1 x F3	76.54 ^b ±0.34	90.43 ^{bc} ±0.37	75.36 ^{bc} ±1.00	34.50 ^{ab} ±1.06	73.73 ^b ±0.58
(T4) B2 x F1	76.73 ^b ±1.17	90.92 ^{bc} ±0.64	76.90 ^{abc} ±2.28	32.48 ^b ±2.32	73.69 ^b ±1.15
(T5) B2 x F2	77.63 ^b ±0.89	89.91 ^c ±0.84	76.12 ^{bc} ±3.31	34.45 ^{ab} ±4.41	75.67 ^b ±1.03
(T6) B2 x F3	81.28 ^a ±1.03	91.95 ^{ab} ±0.23	83.78 ^a ±0.48	39.76 ^{ab} ±4.86	79.12 ^a ±1.39
(T7) B3 x F1	78.24 ^b ±0.77	90.74 ^{bc} ±0.67	80.15 ^{ab} ±0.96	33.76 ^{ab} ±0.76	75.62 ^b ±1.43
(T8) B3 x F2	81.03 ^a ±0.41	91.75 ^b ±0.48	77.50 ^{abc} ±2.50	39.90 ^{ab} ±0.90	79.96 ^a ±0.91
(T9) B3 x F3	81.87 ^a ±0.61	93.31 ^a ±0.17	83.58 ^a ±1.25	41.98 ^a ±1.75	79.43 ^a ±0.77

a,b,c: Means in the same column with different letters are significantly different (p<0.05)

Regarding to methionine levels the growth performance at the same level of betaine, folic acid or interaction between them improved with Exp. II (0.45% methionine) than Exp. I (0.33% methionine). These results were in agreement with those of Attia *et al.* (2005) who reported that means of chick performance was significantly improved linearly by increasing dietary methionine level during all experimental periods from 1 to 56 days of age. Likewise, Vazquez-Anon *et al.* (2006) stated that performance improved at all times for most parameters after supplementing with DLM (p<0.05). Methionine may act as a lipotropic agent through its role as an amino acid in balancing protein, or through its role as a methyl donor and involvement in choline, betaine, folic acid and vitamin B12 metabolism (Tillman and Pesti, 1986).

Digestion coefficient

Experiment I: Digestion Coefficient of nutrients at low level of methionine (Exp I) are listed in Table 5. The digestion coefficients of OM, CP, EE, CF and NFE were significantly (p>0.05) increased with increasing betaine or folic acid levels in diets, except of betaine effects on CF digestibility. These results are in agreement with the previous results of body weight and weight gain which increased by increasing of betaine or folic acid level in diets. Significant interactions between betaine×folic acid on digestibility were noted, where, no significant different for increase levels of folic acid at the low level of betaine, while the digestibilities were significantly increased (p>0.05) by increasing folic acid levels at the other levels of betaine.

Experiment II: The effects of betaine and folic acid supplementation at normal level of methionine (Exp.II) on nutrients digestibility are tabulated in Table 6. The

different levels of betaine had no significant influence on OM, CP, EE and NFE digestibility. A significant different (p<0.05) in CF digestibility and ranged between 40.88±1.23 and 43.91±0.71 for T1 and T2, respectively. No significant effects of the different levels of folic acid on CP, EE and CF digestibility. However, significant interactions between betaine and folic acid supplementation for OM, EE, CF and NFE digestibility, therefore, the values increased by increasing folic acid levels at the same level of betaine. The chicks fed different levels of betaine and folic acid had no significant effect on CP digestibility. The same was observed by Attia *et al.* (2005). Regarding to nutrients digestion coefficients for diets containing folic acid levels with no significant differences for CP, EE and CF digestibility. The trend goes parallel with those reported by El-Husseiny *et al.* (2005).

The digestion coefficients of birds fed diets containing the normal level of methionine (Exp. II) were higher than those of (Exp. I). These results were in agreement with those of Naulia and Singh (2002) who noted that the digestibility of DM and OM were significantly higher (p<0.05) on 0.323% methionine level compared to 0.248 or 0.267% level.

Blood plasma parameters: Blood plasma for experimental treatments I and II are recorded in Table 7 and 8. Exp. I, A significant difference was observed on plasma TP, GLO and T. lipids which increased by increasing betaine levels, however, plasma T. lipids decreased and ALT increased linearly with folic acid level. Significant interactions in all plasma parameters except for ALB. Exp. II, Betaine supplementation had no significant effect on most of plasma constituents except for T.P where a significant increase was noticed by

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Table 6: Effect of different levels of betaine and folic acid on nutrients digestibility at normal level of methionine, 0.45% (Exp. II)

Item	Digestibilities %				
	OM	CP	EE	CF	NFE
Betaine (B) effects					
B1 (0.50 gm/kg)	82.23±0.65	93.98±0.36	81.41±0.90	40.88 ^b ±1.23	80.24±0.83
B2 (0.75 gm/kg)	82.46±0.33	93.46±0.84	82.74±1.03	43.91 ^a ±0.71	80.37±0.59
B3 (1.00 gm/kg)	82.64±0.34	93.07±0.48	82.18±0.02	41.17 ^{ab} ±0.00	81.14±0.43
Folic acid (F) effects					
F1 (0.50 mg/kg)	81.36 ^b ±0.45	93.23±0.48	81.04±0.88	40.84±1.07	79.17 ^b ±0.64
F2 (0.75 mg/kg)	82.58 ^a ±0.32	93.75±0.43	80.88±0.71	42.32±1.24	80.92 ^a ±0.47
F3 (1.00 mg/kg)	83.38 ^a ±0.31	93.50±0.34	84.41±0.77	42.81±0.77	81.66 ^a ±0.49
B x F effects					
(T1) B1 x F1	80.11 ^b ±0.86	93.44±0.53	80.04 ^c ±2.10	38.16 ^b ±1.68	77.33 ^b ±1.13
(T2) B1 x F2	83.39 ^a ±0.51	94.48±0.62	81.82 ^b ±0.99	42.08 ^{ab} ±2.77	81.82 ^a ±0.33
(T3) B1 x F3	83.19 ^a ±0.83	94.00±0.80	82.37 ^{abc} ±1.71	42.42 ^{ab} ±1.43	81.56 ^a ±0.72
(T4) B2 x F1	81.95 ^a ±0.49	93.32±0.30	80.40 ^b ±1.58	43.43 ^{ab} ±1.10	80.06 ^a ±0.98
(T5) B2 x F2	82.20 ^a ±0.70	93.65±1.13	81.51 ^b ±0.47	45.20 ^a ±0.23	79.99 ^a ±1.28
(T6) B2 x F3	83.22 ^a ±0.40	93.41±2.65	86.31 ^a ±0.49	43.11 ^{ab} ±1.86	81.07 ^a ±1.09
(T7) B3 x F1	82.03 ^a ±0.55	92.92±1.51	82.69 ^{abc} ±0.45	40.92 ^{ab} ±1.66	80.11 ^a ±0.33
(T8) B3 x F2	82.16 ^a ±2.50	93.12±0.36	79.30 ^b ±1.70	39.67 ^{ab} ±1.75	80.96 ^a ±1.42
(T9) B3 x F3	83.72 ^a ±0.45	93.19±0.59	84.56 ^{ab} ±0.38	42.92 ^{ab} ±1.19	82.35 ^a ±0.88

a,b,c,d: Means in the same column with different letters are significantly different (p<0.05)

Table 7: Effect of different levels of betaine and folic acid on some blood parameters at low level of methionine,0.33% (Exp. I)

Item	Blood parameters							
	T.P	ALP.	GLO.	A/G	T.LIPS.	CHOL.	AST	ALT
Betaine (B) effects								
B1 (0.50 gm/kg)	3.62 ^a ±0.08	0.88±0.05	2.73 ^a ±0.07	0.33±0.02	0.29 ^a ±0.01	120.74±6.96	26.76±0.69	12.03±0.69
B2 (0.75 gm/kg)	3.90 ^a ±0.13	0.83±0.05	3.07 ^a ±0.14	0.28±0.02	0.33 ^{ab} ±0.02	121.12±3.19	26.42±0.81	12.23±0.43
B3 (1.00 gm/kg)	3.59 ^a ±0.09	0.86±0.04	2.78 ^a ±0.08	0.32±0.02	0.36 ^a ±0.02	137.16±5.02	25.23±0.60	11.80±0.48
Folic acid (F) effects								
F1 (0.50 mg/kg)	3.63±0.07	0.85±0.03	2.77±0.06	0.31±0.01	0.37 ^a ±0.02	122.97±5.23	26.38±0.16	11.26 ^a ±0.49
F2 (0.75 mg/kg)	3.76±0.15	0.88±0.05	2.88±0.17	0.32±0.03	0.31 ^a ±0.01	130.92±7.35	26.72±0.71	12.10 ^a ±0.54
F3 (1.00 mg/kg)	3.72±0.11	0.84±0.05	2.88±0.06	0.29±0.02	0.31 ^a ±0.03	123.64±5.97	25.31±0.61	12.71 ^a ±0.49
B x F effects								
(T1) B1x F1	3.69 ^a ±0.07	0.85±0.06	2.85 ^a ±0.03	0.30 ^a ±0.02	0.33 ^{ab} ±0.01	109.53 ^b ±9.84	27.17 ^{ab} ±1.96	10.25 ^a ±0.23
(T2) B1x F2	3.55 ^a ±0.06	1.00±0.03	2.55 ^a ±0.09	0.40 ^a ±0.03	0.28 ^a ±0.02	127.77 ^{ab} ±18.90	26.73 ^{ab} ±0.72	11.87 ^{ab} ±1.31
(T3) B1x F3	3.60 ^a ±0.26	0.80±0.10	2.80 ^a ±0.16	0.28 ^a ±0.02	0.27 ^a ±0.02	124.93 ^{ab} ±5.49	26.37 ^{ab} ±1.12	13.99 ^a ±0.69
(T4) B2x F1	3.59 ^a ±0.22	0.82±0.04	2.77 ^a ±0.18	0.29 ^a ±0.00	0.38 ^{ab} ±0.04	123.07 ^{ab} ±5.20	25.70 ^{ab} ±1.46	11.62 ^{ab} ±0.99
(T5) B2x F2	4.30 ^a ±0.11	0.81±0.14	3.49 ^a ±0.23	0.24±0.05	0.29 ^b ±0.02	116.90 ^{ab} ±4.90	27.53 ^a ±1.71	12.58 ^{ab} ±2.19
(T6) B2x F3	3.82 ^a ±0.06	0.86±0.06	2.96 ^a ±0.01	0.29 ^a ±0.02	0.32 ^{ab} ±0.02	123.41 ^{ab} ±7.59	24.57 ^{ab} ±0.38	10.92 ^{ab} ±0.66
(T7) B3x F1	3.60 ^a ±0.07	0.90±0.06	2.70 ^a ±0.11	0.34 ^{ab} ±0.04	0.41 ^a ±0.03	136.30 ^{ab} ±4.98	26.27 ^{ab} ±1.18	11.92 ^a ±1.39
(T8) B3x F2	3.44 ^a ±0.15	0.83±0.02	2.61 ^a ±0.14	0.32 ^{ab} ±0.01	0.35 ^{ab} ±0.02	148.09 ^a ±4.12	25.90 ^{ab} ±0.31	11.93 ^{ab} ±1.13
(T9) B3x F3	3.74 ^a ±0.23	0.85±0.10	2.89 ^a ±0.16	0.29 ^b ±0.03	0.33 ^{ab} ±0.02	122.05 ^{ab} ±13.15	23.53 ^a ±0.78	11.55 ^{ab} ±0.69

a,b,c,: Means in the same column with different letters are significantly different (p<0.05)

Table 8: Effect of different levels of betaine and folic acid on some blood parameters at normal level of methionine,0.45% (Exp. II)

Item	Blood parameters							
	T.P	ALP.	GLO.	A/G	T.LIPS.	CHOL.	AST	ALT
Betaine (B) effects								
B1 (0.50 gm/kg)	3.90 ^{ab} ±0.22	0.96±0.05	2.94±0.17	0.33±0.01	0.33±0.03	141.10±13.29	25.14±0.96	11.77±0.75
B2 (0.75 gm/kg)	3.77 ^b ±0.15	0.96±0.06	2.81±0.12	0.35±0.02	0.34±0.02	151.19±05.85	25.14±1.08	12.13±0.85
B3 (1.00 gm/kg)	4.33 ^a ±0.22	1.09±0.04	3.24±0.22	0.35±0.02	0.31±0.02	141.32±8.08	24.17±0.51	10.54±0.60
Folic acid (F) effects								
F1 (0.50 mg/kg)	4.27 ^a ±0.24	0.97±0.06	3.30 ^a ±0.19	0.30 ^a ±0.01	0.32±0.03	137.39±10.05	24.22±0.90	10.87±0.64
F2 (0.75 mg/kg)	4.12 ^a ±0.17	1.09±0.05	3.02 ^a ±0.16	0.37±0.02	0.33±0.02	145.34±08.48	25.81±1.00	12.27±0.84
F3 (1.00 mg/kg)	3.62 ^a ±0.17	0.95±0.04	2.67 ^a ±0.14	0.36±0.01	0.33±0.02	144.54±05.4	24.82±0.5	11.48±0.43
B x F effects								
(T1) B1x F1	4.29 ^{ab} ±0.48	1.00 ^{ab} ±0.15	3.29 ^{ab} ±0.03	0.30 ^a ±0.02	0.24 ^a ±0.03	126.33±23.75	23.73±2.25	10.98 ^{ab} ±1.18
(T2) B1x F2	4.09 ^{ab} ±0.15	1.01 ^{ab} ±0.04	3.08 ^{ab} ±0.15	0.33 ^{ab} ±0.02	0.40 ^{ab} ±0.02	130.83±23.5	26.90±1.6	11.02 ^{ab} ±1.68
(T3) B1x F3	3.32 ^a ±0.23	0.87 ^b ±0.06	4.46 ^a ±0.18	0.35 ^{ab} ±0.01	0.34 ^{ab} ±0.05	166.13±22.9	24.80±0.76	13.31 ^{ab} ±0.82
(T4) B2x F1	3.65 ^{ab} ±0.10	0.83 ^b ±0.02	2.81 ^a ±0.39	0.30 ^a ±0.01	0.40 ^{ab} ±0.01	160.97±5.59	24.97±1.77	11.32 ^{ab} ±1.72
(T5) B2x F2	3.88 ^{ab} ±0.32	1.10 ^{ab} ±0.13	2.78 ^a ±0.23	0.40±0.04	0.30 ^{cd} ±0.03	157.10±11.28	25.90±2.77	14.17±0.76
(T6) B2x F3	3.80 ^{ab} ±0.38	0.96 ^{ab} ±0.04	2.84 ^a ±0.34	0.34 ^{ab} ±0.03	0.31 ^{ab} ±0.01	135.50±8.01	24.57±1.71	10.92 ^{ab} ±1.39
(T7) B3x F1	4.88 ^a ±0.22	1.08 ^{ab} ±0.06	3.80 ^a ±0.26	0.29 ^a ±0.03	0.32 ^{ab} ±0.02	124.87±14.10	23.97±1.04	10.32 ^{ab} ±0.63
(T8) B3x F2	4.39 ^{ab} ±0.40	1.17 ^a ±0.05	3.21 ^a ±0.41	0.38 ^{ab} ±0.05	0.28 ^d ±0.03	148.10±1.72	24.63±0.77	11.63 ^{ab} ±1.48
(T9) B3x F3	3.73 ^{ab} ±0.29	1.03 ^{ab} ±0.09	2.70 ^a ±0.20	0.38 ^{ab} ±0.01	0.33 ^{ab} ±0.04	151.00±19.4	23.90±1.14	9.67 ^a ±0.88

a,b,c,d: Means in the same column with different letters are significantly different (p<0.05)

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Table 9: Effect of different levels of betaine and folic acid on economic efficiency of the experimental diets for Exp. I & II

Experimental diet	Input			Output			
	F l/chick (kg)	Price/kg feed (LE)	Cost of feed (LE)	Body weight/ chick (kg)	Price/ chick (LE)	Net revenue (N.R.) (LE)	E.Ef
----- Experiment I (0.33% Methionine) -----							
(T1) B1xF1	3.242	1.490	4.83	1.630	9.45	4.45	0.96
(T2) B1xF2	3.088	1.491	4.60	1.668	9.67	5.07	1.10
(T3) B1xF3	3.126	1.492	4.66	1.680	9.74	5.08	1.09
(T4) B2xF1	3.002	1.491	4.48	1.665	9.66	5.18	1.16
(T5) B2xF2	3.056	1.492	4.56	1.716	9.95	5.39	1.18
(T6) B2xF3	3.087	1.493	4.61	1.768	10.25	5.64	1.22
(T7) B3xF1	3.014	1.492	4.50	1.689	9.80	5.30	1.17
(T8) B3xF2	3.054	1.493	4.56	1.754	10.17	5.67	1.23
(T9) B3xF3	3.071	1.494	4.59	1.799	10.43	5.89	1.27
----- Experiment II (0.45% Methionine) -----							
(T1) B1xF1	3.117	1.518	4.73	1.715	9.95	5.22	1.10
(T2) B1xF2	3.189	1.519	4.84	1.799	10.43	5.59	1.16
(T3) B1xF3	3.096	1.520	4.71	1.797	10.42	5.71	1.21
(T4) B2xF1	3.037	1.519	4.61	1.740	10.09	5.48	1.19
(T5) B2xF2	3.101	1.520	4.71	1.799	10.43	5.72	1.21
(T6) B2xF3	3.233	1.521	4.92	1.767	10.25	5.33	1.08
(T7) B3xF1	3.091	1.520	4.70	1.785	10.35	5.65	1.20
(T8) B3xF2	3.124	1.521	4.75	1.785	10.35	5.60	1.18
(T9) B3xF3	3.101	1.522	4.72	1.823	10.57	5.85	1.24

Price of chick = Average of body weight/kg×price of one kg of body weight (6.5 LE), Net revenue = Price of chick/LE-Cost of feed/LE

increasing betaine level. Likewise, folic acid levels had significant decrease on T.P and GLO values versus A/G ratio which increased by graded levels. Most of parameters had significant interactions while, CHOL and AST had no significant interactions. In general, the results were nearly similar to that reported by Attia *et al.* (2005). Concerning methionine effect, birds fed diets containing the normal level of methionine in Exp. II had higher values of blood plasma than those in Exp. I. These results were similar to those of Attia *et al.* (2005) who stated that concentration serum AST and ALT decreased with increasing the level of supplemental methionine. Liver transaminases, AST and ALT are essential in protein biosynthesis (El-Ansary *et al.*, 1996) and the decrease in their concentration with increasing level of methionine supplementation reflects better liver function.

Economic efficiency: The economic efficiency of experimental treatments are recorded in Table 9. The net revenue and economic efficiency values ranged between 4.62-5.89 (LE) and 0.96-1.27 (Exp. I) or varied from 5.22-5.85 (LE) and 1.08-1.24 (Exp. II), respectively. The lowest values were recorded for T1 and T6 of Exp. I and II, respectively, while the highest values were listed for T9 for either experiment I or II.

It may be concluded that the graded levels of betaine supplemented to diets containing low level of methionine improved the chick performance and economic efficiency, the improvement depressed when chicks received methionine recommended. While, the use of different levels of folic acid improved the

performance parameters and economic efficiency when diets contained low or recommended level of methionine.

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