

BETAINE AND CHOLINE IN BROILER DIETS

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ABSTRACT

An experiment was conducted: 1) to compare the response of broiler chicks fed diets supplemented with betaine or choline on an equi-molar basis and 2) to examine the effect of using higher levels of supplemental betaine on broiler chicks performance. A total number of 240 unsexed day-old HUBBARD broiler chicks were distributed into 6 treatments of 40 chicks each in four replicates (10 chicks per replicate). The diets were formulated to contain about 21.6% and 17.7% CP and about 2971 and 3057 Kcal ME/Kg feed at starter and finisher period, respectively. The control (T1) diets were supplemented with 690 ppm choline chloride (50%) to supply 300 ppm choline (according to Egyptian recommendations). The chicks of treatment 2 (T2) were fed diets supplemented with 175 ppm of commercial betaine (91%) to supply an equivalent amount of molecules that choline furnished in control diets. The birds of treatment 3 (T3) and 4 (T4) were fed diets supplemented with choline chloride (50%) or betaine (91%) at levels of 1650 or 420 ppm, respectively to supply the recommended level of choline (715 ppm) to "HABBARD" broilers . However, treatments 5 (T5) and 6 (T6) were fed diets containing higher levels of betaine (665 ppm for T5 and 910 ppm for T6).

The results of entire experimental period revealed that the values obtained with respect to the performance of chicks and carcass characteristics were nearly similar.

Under the conditions of this experiment, it could be concluded that:

- 1- Betaine can effectively replace choline in broiler feed whilst maintaining performance.
- 2- On an equi-molar basis, 1 kg betaine (91%) can replace about 4 kg choline chloride (50%).
- 3- Using betaine (91%) up to 910 ppm in broiler diets gave no extra benefit, as long as these diets were formulated to contain enough essential amino acids and birds reared under no stress conditions.

INTRODUCTION

Betaine (glycine betaine, trimethylamine) is a non-toxic amino acid derivative found widely distributed in feedstuffs and can serve as a methylating agent (Bird *et al.*, 1966). Vertebrates are unable to synthesize methyl groups, and thus need to receive them in the diet (Kidd *et al.*, 1997). Betaine, choline and methionine are the three main dietary sources of methyl groups in farm animal diets. However, the methyl groups of dietary methionine is needed for protein synthesis (Smolin and Benevega, 1989) and choline is predominantly used in cell membranes (Davies *et al.*, 1992., Rompala and King, 1995 and Stryer, 1995). Of the three compounds, only betaine can act directly as a methyl group donor (Kettunen *et al.*, 2001). The methyl groups of choline become available when choline is oxidized to betaine in a two- steps enzymatic reaction, taking place in the mitochondria of liver cells. Choline is oxidized to betaine aldehyde, which is then converted to betaine by the enzyme system choline oxidase (Zeisel, 1981). Emmert *et al.* (1996) showed that, although inefficient as a methyl donor in choline biosynthesis, methionine can replace one function of choline, which is

donation of a methyl group to the single-carbon pool. Betaine, a product of choline oxidation, can also replace this function. However, choline *per se* is needed for phospholipids and acetyl-choline synthesis, which have priority over the need for methyl groups. It has been shown that addition of betaine to a choline-free purified diet will not produce a growth response until approximately two-thirds of the choline requirement has been furnished by choline *per se* (Lowry *et al.*, 1987). The limited synthesis of choline via S-adenosylmethionine methylation and the irreversible nature of choline oxidation to betaine mean that a diet severely deficient in choline should not show a growth response to methionine or betaine, whereas a diet marginally deficient in choline will probably respond to choline, betaine or methionine (Emmert *et al.*, 1996). Betaine may also have a role in intestinal osmoprotection (Kidd *et al.*, 1997). The intestinal epithelium of chickens encounters hyperosmotic luminal fluids during the process of digestion (Mongin, 1976) and dietary betaine has been found to aid in situations where the health of the intestine is compromised (Ferket, 1995; Augustine *et al.*, 1997; Allen *et al.*, 1998 and Waldenstedt *et al.*, 1999). Abel *et al.* (1985) has suggested that feed efficiency is improved more by adding betaine to the diet than by adding choline. However, others did not find an improvement (Lowry *et al.*, 1987 and Schutte *et al.*, 1997). Also, Harms and Russell (2002) concluded that the addition of betaine does not increase performance of commercial layers when compared to choline or fed in combination with choline. On the other hand, Simon (1999) showed that the results of growth performance of broiler chicks fed on diets supplemented with betaine were inconsistent. The author concluded that, "the suggestion that betaine is able to decrease fattening remains to be demonstrated" and "the slimming effect of betaine is very doubtful".

Choline can also be used as methyl donor in animal feeds. In poultry, methyl groups are available after the conversion to betaine in the liver. However, dietary betaine is twice as efficient as the equi-molar dietary choline for increasing liver betaine levels in broiler chick (Saarinen *et al.*, 2001).

The objectives of this study were:

- 1- to compare the response of broiler chicks fed diets supplemented with betaine or choline on equi-molar bases.
- 2- to examine the effect of using higher levels of supplemental betaine on broiler chicks performance.

MATERIALS AND METHODS

This experiment was carried out at poultry research farm , Poultry Production Dept. , Fac. of Agriculture, Ain-Shams University, Cairo,Egypt.

A number of 240 unsexed, day-old "Hubbard" broiler chicks were used in this experiment. The birds were wing-banded and reared in battery chicks that located in an open-sided house. The chicks were randomly divided equally into 6 treatments of 40 chicks each in four replicates with 10 chicks each having nearly similar body weight. Feed and water were provided *ad libitum* and a 24 hours artificial light was maintained. Chicks were kept under similar conditions of management throughout the experimental period which lasted for 49 days. Diets were formulated to contain about 21.6% CP and 2971 Kcal.ME/Kg. feed, for starter/grower period and 17.7% CP and 3057

Kcal.ME/Kg.feed, for finisher period (according to Egyptian recommendations). The control treatment (T₁) diets were supplemented with 690 mg of choline chloride (50%) per kg diet to supply 300 ppm choline (the Egyptian recommendation for supplemental choline is 250 ppm as a minimum for broilers). However, the chicks of treatment 2 (T₂) were fed diets supplemented with 175 mg of commercial betaine (91%) per kg diet to supply an equivalent amount of molecules that choline furnished in control diets. The birds of treatments 3 (T₃) and 4 (T₄) were fed diets supplemented with choline chloride (50%) or betaine (91%) at levels of 1650 mg/kg or 420 mg/kg, respectively to meet the recommendation of "Hubbard" broiler for supplemental choline (715 ppm). On the other hand, treatments 5 (T₅) and 6 (T₆) were fed diets containing higher levels of betaine than T₄ (665 mg/kg for T₅ and 910 mg/kg for T₆). The composition and calculated analysis of the experimental diets are shown in Tables (1) and (2). At the end of the 4th and 7th weeks of age, chicks were individually weighed, feed intake values were measured and feed conversion ratio values were calculated.

Table 1. The composition and calculated analysis of the experimental diets: Starter / Grower (0-28 days of age).

Ingredients %	Treatments					
	T1	T2	T3	T4	T5	T6
Yellow corn	65.50					
Soybean meal (44%)	24.50					
Meat meal (62%)	6.65					
Fish meal (72%)	1.50					
Bone meal	1.01	1.0615	1.00	1.0370	1.0125	1.0120
Lime stone	0.10	0.10	0.034	0.10	0.10	0.0760
DL.methionine	0.14					
NaCl	0.30	0.30	0.28	0.30	0.30	0.30
Vit. & Min. mix.*	0.231					
Choline chloride (50%)	0.0690	-	0.1650	-	-	-
Betaine (91%)	-	0.0175	-	0.0420	0.0665	0.0910
Total	100	100	100	100	100	100
Calculated analysis : **						
Crude protein %	21.63					
ME (k.cal. / kg diet)	2971					
Calcium %	0.93	0.94	0.90	0.94	0.93	0.92
Available Phosphorus %	0.47	0.48	0.47	0.47	0.47	0.47
Methionine %	0.50					
Methionine + Cystine %	0.84					
Lysine %	1.14					
Na %	0.18					
EE %	3.12					
CF %	3.23					
Added choline (ppm)	300	300	715	715	1134	1552
Dietary choline (ppm)	1310					
Total choline equivalent (ppm)	1609	1609	2025	2025	2444	2862

* Vitamin & Mineral mixture supplied per Kg of diet: Vit A, 12000 I.U; Vit D₃, 2500 I.U; Vit E, 30 mg; Vit K₃, 3 mg; Vit B₁, 1 mg; Vit B₂, 5 mg; Vit B₆, 1.5mg; Vit B₁₂, 12µg; Niacin, 30 mg; Pantothenic acid, 10 mg; Folic acid, 1 mg; Biotin, 50µg; , Copper, 4 mg; Iodine, 1 mg; Iron, 30 mg; Manganese, 60 mg; Zinc, 50 mg, Cobalt, 0.1 mg and Selenium, 0.3 mg.

** Calculated based on feed composition Tables of NRC (1994)

Table 2: The composition and calculated analysis of the experimental diets: Finisher (29 - 49 days of age).

Ingredients %	Treatments					
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
Yellow corn	75.00					
Soybean meal (44%)	13.00					
Meat meal (62%)	6.65					
Fish meal (72%)	1.50					
Wheat bran	2.00					
Bone meal	1.02					
lime stone	0.09	0.1415	0.034	0.1570	0.1325	0.1080
DL.methionine	0.10					
NaCl	0.34	0.34	0.30	0.30	0.30	0.30
Vit. & Min. mix.*	0.231					
Choline chloride (50%)	0.0690	-	0.1650	-	-	-
Betaine (91%)	-	0.0175	-	0.0420	0.0665	0.0910
Total	100	100	100	100	100	100
Calculated analysis:**						
Crude protein %	17.67					
ME (k.cal. / kg diet)	3057					
Calcium %	0.90	0.94	0.90	0.94	0.93	0.92
available Phosphorus %	0.45	0.48	0.47	0.47	0.47	0.47
Methionine %	0.41					
Methionine + Cystine %	0.69					
Lysine %	0.87					
Na %	0.19					
EE %	3.45					
CF %	2.90					
Added choline (ppm)	300	300	715	715	1134	1552
Dietary choline (ppm)	1072					
Total choline equivalent (ppm)	1371	1371	1787	1787	2206	2624

* Vitamin & Mineral mixture supplied per Kg of diet: Vit A, 12000 I.U; Vit D₃, 2500 I.U; Vit E, 30 mg; Vit K₃, 3 mg; Vit B₁, 1 mg; Vit B₂, 5 mg; Vit B₆, 1.5mg; Vit B₁₂, 12µg; Niacin, 30 mg; Pantothenic acid, 10 mg; Folic acid, 1 mg; Biotin, 50µg; , Copper, 4 mg; Iodine, 1 mg; Iron,30 mg; Manganese, 60 mg; Zinc, 50 mg, Cobalt, 0.1 mg and Selenium, 0.3 mg.

** Calculated based on feed composition Tables of NRC (1994)

At the end of the 7th week of age, all birds were deprived from feed for 12 hrs. and sample of 8 birds from each treatment (two birds from each replicate) were weighed and slaughtered to evaluate the carcass characteristics. Carcass and giblets (liver, gizzard and heart) were weighed and abdominal fat including fat of abdominal cavity as well as fat surrounding the gizzard was removed and weighed. These data were presented as percentage of live body weight.

Data were statistically analyzed by using the linear model (SX, 1992). A simple one- way classification analysis followed by LSD test were used to examine the differences between means.

RESULTS AND DISCUSSION

The productive performance of broiler chicks fed the different dietary treatments as live body weight, feed consumption and feed conversion ratio during the starting / growing period (0-28 days of age), the finishing period (29-49 days of age) and overall the experimental period (0 to 49 days of age) are summarized in Table (3).

The results at 28 days of age showed that, the live body weight of chicks increased ($P>0.05$) with increasing the level of dietary choline by additional choline chloride (T_1 and T_3) or betaine (T_2 , T_4 , T_5 and T_6). The obtained feed consumption values of betaine treatments (T_2 and T_4) were slightly lower ($P>0.05$) than choline treatments (T_1 and T_3). It must be mentioned that the feed conversion ratio improved ($P> 0.05$) during starting/ growing period with replacement choline by betaine.

Table 3. The performance of broiler chicks as affected by the experimental diets.

Item	Treatments						SEM *	P **
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆		
at the 28th day of age :								
Live body weight (g/bird)	685.8	697.6	719.2	729.9	740.9	732.4	53.59	0.892
Feed intake (g / bird)	1114	1087	1165	1154	1162	1171	74.59	0.839
Feed conversion ratio	1.627	1.558	1.623	1.584	1.574	1.600	0.0345	0.330
at the 49th day of age :								
Live body weight (g bird)	1836	1835	1841	1851	1886	1846	88.36	0.992
Feed intake (g / bird)	3490	3453	3504	3503	3574	3530	196.09	0.994
Feed conversion ratio	1.900	1.880	1.898	1.894	1.900	1.911	0.051	0.994

* Standard error mean for comparison.

** Probability.

Results obtained during the entire period which lasted for 49 days of age, revealed that the values obtained with respect to the performance of chicks for body weight, feed consumption and feed conversion ratio were nearly similar and there was no clear trend due to the different treatments. These results suggest that betaine could substitute choline in broiler diets without any adverse effect on growth performance.

These results are in agreement with those reported by Sakomura *et al.* (1996) who examined the use of betaine feed grade in reducing concentration of choline in broiler diets. The treatments tested levels of supplementation of choline and betaine. They found that betaine could substitute choline in all tested diets and feed conversion ratio was not affected by treatments. Pesti *et al.* (1980, 1981) demonstrated that betaine and choline appear equal as sources of methyl groups. Yao and Vance (1989) supported the hypothesis that betaine functions to spare choline for the formation of phospholipids.

It seems likely that, under the conditions of this experiment, using supplemental levels of betaine (91%) up to 910 ppm in broiler diets, either during starter or finisher period, gave no extra benefit on growth performance (Table 3). These findings are in agreement with the results of Mcdevitt *et al.* (2000) who used three levels of supplemental betaine in growing diets (369,

448 or 646 ppm) and finishing diets (352, 367 or 271 ppm). They found no significant effect of adding betaine levels in broiler chicks diet during grower and finisher periods. Kidd *et al.* (1997) concluded that, poultry do not have a specific requirement for betaine provided they consume or synthesize sufficient choline. However, supplemental betaine may be advantageous during certain physiologically challenging conditions, including the high metabolic demand of rapid growth, diseases and osmotic stress in different cell types. Halpin and Baker (1984) and Lowry *et al.* (1987) reported that because diets were formulated to be adequate in all essential nutrients, including methionine, cystine and choline, differences in growth variables were not expected.

In the present study, it is observed that the diets have adequate choline, coming from ingredients (Tables 1 and 2) as listed in NRC (1994) broiler chicks requirements (1300 and 1000 ppm for starting and finishing period, respectively). The results of this experiment support earlier research where scientists have looked at whether betaine can replace choline. In experiment with broilers fed maize-soy diets devoid of supplemental choline, Miles *et al.* (1987) showed that adding 1.1 gm/kg choline or 1.06 gm/kg betaine improved growth, but no further growth resulted from adding both compounds (choline and betaine). Birds grew faster when the basal diet was supplemented with 2.4 gm/kg DL-methionine either alone or in combination with choline or betaine. They concluded that betaine may substitute for choline. Kidd *et al.* (1997) reported that the methylation efficiency of betaine and methionine are similar, but choline is a less efficient methyl donor. They added that on a practical basis, 1kg of anhydrous betaine (97%) will provide the equivalent amount of methyl groups as 1.65 kg of choline chloride (70%).

Methionine, choline and betaine are sources of labile methyl groups and play an important role in methylation reactions. A key reaction is the methylation of homocysteine to methionine following transfer of a methyl group from choline. However, in order to act in this capacity, choline must first be converted into betaine. There is some evidence that the provision of either supplemental choline (Pesti *et al.*, 1981) or betaine (Virtanen and Rosi, 1995) in the diet can spare methionine from this cycle and increase its availability as an essential amino acid and for protein synthesis. Harms *et al.*, (1990) reported that hens would respond to choline supplementation of diet marginal in methionine, however, when the diet contained enough methionine, no supplemental choline was needed. These findings supported the present results as the methionine levels of diets in the present study were adequate to supply the chick requirements for protein synthesis, so, no supplemental choline or betaine was needed.

The effect of different treatments on carcass traits as percentages of live body weight is shown in Table (4). Dressing percentage values of chicks fed diets supplemented with choline chloride (T₁ and T₃) were not significantly different of that fed diets supplemented with betaine (T₂, T₄, T₅ and T₆). Although these differences were not statistically significant, there are increases in dressing percentage with replacement of choline chloride by betaine. The average values of abdominal fat percentage were decreased with increasing the level of supplemental either choline or betaine. Also, the abdominal fat percentage was decreased with replacement choline chloride by betaine. There were no significant differences among treatments due to

either choline or betaine levels. Giblets percentages (expressed as the total of gizzard, liver and heart) were nearly similar and there was no clear trend due to the different treatments.

Table 4. Carcass characteristics of broiler chicks at 49th day of age as affected by the experimental diets.

Item	Treatments						SEM *	P **
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆		
Live body weight (g / bird)	1836	1835	1841	1851	1886	1846	88.36	0.992
Dressing %	72.81	74.53	72.85	74.78	74.14	73.36	0.977	0.228
Abdominal fat %	2.69	2.51	2.57	2.41	2.38	2.37	0.236	0.718
Giblets %	5.06	5.18	5.12	5.11	5.06	5.09	0.085	0.730
Total edible parts %	77.87	79.70	77.96	79.89	79.20	78.45	0.962	0.193

* Standard error mean for comparison.

** Probability.

These results are in agreement with those reported by Deng and Wong (1997) who found that the addition of 0.05% methionine or betaine did not affect net dressing percentage, breast meat, leg meat or abdominal fat of the groups supplemented with betaine. Florou-Paneri *et al.* (1997) reported that, at the end of the finishing period, partial substitution (80%) of betaine for supplementary choline in the diets of broiler chicks did not significantly influence body weight gain or carcass characteristics (carcass weight and yield, liver and gizzard weight and abdominal fat). Their basal diet, were formulated to contain 1675 ppm choline at starting period and 1575 ppm at finishing period by supplementing with choline at level of 560 ppm all over the experimental period. However, in the study of McDevitt *et al.* (2000) addition of betaine to methionine-supplemented diet significantly decreased abdominal fat pad size. Bell (1995) reported a 12% decrease in back fat thickness in swine fed a diet supplemented with betaine. Soundreson and Mackinlay (1990) observed a reduction in the carcass fat of chicks fed a basal diet supplemented with betaine and methionine combined compared with basal diet supplemented with choline and methionine combined. Esteve-Garcia and Mack (2000) found that betaine significantly improved carcass yield.

It could be concluded that, under the conditions of this experiment:

- 1) Betaine can effectively replace choline in broiler feed whilst maintaining performance.
- 2) On an equi-molar basis, 1 kg betaine (91 %) can replace about 4 kg choline chloride (50%).
- 3) Using betaine (91 %) up to 910 ppm in broiler diets gave no extra benefit, as long as broiler diets is formulated to contain enough essential amino acids and the birds reared under no stress conditions.

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البيتاين والكولين في علائق دجاج اللحم.

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أجريت تجربة: (١) لمقارنة استبدال الكولين بالبيتاين في علائق كتاكيت اللحم، على أساس المكافئ الجزيئي، (٢) دراسة تأثير استخدام مستويات أعلى من البيتاين على الأداء الإنتاجي لدجاج اللحم.

استخدم في هذه التجربة عدد ٢٤٠ كتكوت "هبرد" عمر يوم وزعت على ٦ معاملات بكل منها ٤٠ كتكوت موزعة على أربع مكررات (١٠ كتاكيت في كل مكرر). تم تغذية الطيور على علائق متساوية في البروتين (٢١,٦% ، ١٧,٧% بروتين خام) والطاقة (٢٩٧١, ٣٠٥٧ كيلو كالورى / كجم علف) في فترتي البادئ والناهي - على التوالي.

لم تظهر النتائج أي فروق معنوية بين المعاملات المختلفة في معدلات الأداء الإنتاجي سواء في نهاية مرحلة البادئ أو في نهاية التجربة وكذلك كانت قياسات الذبيحة متقاربة بين المعاملات الغذائية المختلفة.

تحت ظروف هذه الدراسة- يمكن استخلاص أن:

- (١) يمكن أن يحل البيتاين محل الكولين في علائق كتاكيت اللحم بدون أي تأثير على معدلات الأداء الإنتاجي وكذا صفات الذبيحة.
- (٢) يمكن أن يستبدل ١ كجم بيتاين (٩١%) بحوالي ٤ كجم كولين كلورايد (٥٠%).
- (٣) استخدام البيتاين بمستوى أعلى حتى ٩١٠ جزء في المليون في تغذية دجاج اللحم لا يحسن الإنتاج طالما أن العلائق محتوية على كميات كافية من الأحماض الأمينية الأساسية، والطيور لم تقع تحت تأثير ظروف الإجهاد المختلفة.