Iranian Journal of Applied Animal Science

The Effect of Choline Chloride Supplementation on Performance Parameters and Carcass Characteristics of Broiler

Research Article

M.E. Hossain[1*], G.B. Das[1], M.M. Hasan[2], A.H. Shaikat[2] and A.S.M. Bari[3]

[1] Department of Animal Science and Nutrition, Faculty of Veterinary Medicine, Chittagong Veterinary and Animal Science University, Khulshi, Chittagong-4225, Bangladesh
[2] Department of Physiology, Pharmacology and Biochemistry, Faculty of Veterinary Medicine, Chittagong Veterinary and Animal Science University, Khulshi, Chittagong-4225, Bangladesh
[3] Department of Pathology, University of Agricultural, Mymensingh, Bangladesh

Received on: 27 Jul 2013
Revised on: 18 Sep 2013
Accepted on: 30 Sep 2013
Online Published on: Jun 2014

*Correspondence E-mail: emrancvasu@yahoo.com

Online version is available on: www.ijas.ir

ABSTRACT

Two hundred and forty Hubbard Classic broiler chicks were used in a 35 day trial at Chittagong Veterinary and Animal Science University farm, Bangladesh to study the effects of supplemental choline chloride on performance parameters, carcass characteristics and their association in commercial broiler. All birds had free access to *ad libitum* feeding. Birds were fed four types of diet i.e. diet without choline chloride (T_0), diet containing 0.1 g / 100 g choline chloride (T_1), 0.2 g / 100 g choline chloride (T_2) and 0.3 g / 100 g choline chloride (T_3). Results indicated that weight gain differed (P<0.05) at 5th week irrespective of the level of supplemental choline. Similar to weight gain, feed intake also differed (P<0.05) at 5th week. However, feed conversion ratio differed from the 3rd to the 5th weeks. Weight gain and feed intake were positively correlated and feed conversion ratio was negatively correlated with carcass parameters. It could therefore be inferred that increasing levels of supplemental choline may progressively improve weight gain, feed efficiency and carcass characteristics in commercial broiler.

KEY WORDS broiler, carcass characteristics, choline chloride, performance parameters.

INTRODUCTION

Choline was first isolated from ox bile in 1849. Its nutritional significance has been recognized since 1930 and now it is a vital dietary supplement for birds (Garrow, 2007; Scott *et al.* 1982). Supplementation of choline in broiler diet improves feed intake (Swain and Johri, 2000), feed efficiency (Baker *et al.* 1999) and weight gain (Baker *et al.* 1983; Pesti *et al.* 1980). Choline acts as a constituent of lecithin to maintain cell structure, normal maturation of cartilage and prevention of perosis (Jukes, 1940). Choline is an emulsifier which mobilizes fat from the liver to cells to prevent abnormal accumulation of fat within hepatocytes for healthy liver and gallbladder (Artom, 1953a; Artom, 1953b; Artom, 1953c). Choline is a precursor for acetyl choline, which acts as a neurotransmitter for the sympathetic nervous system. Despite a wide range of physiological activities, many studies (Artom, 1956; Hudgins *et al.* 2000; Griffith *et al.* 1969; Pearce, 1975; Whitehead and Randall, 1982) show that there are a number of complications, contradictions and uncertainties regarding the specific role of choline, particularly to promote growth and improve carcass parameters. Additionally, these findings in many cases are several decades old which do not address the present scenario of high energy high protein feed formulation, which leads to unusual deposition of abdominal fat in broiler harmful for human health. Therefore, it appears that the role of choline is restricted to a few *in vivo* and *in vitro* dated clinical studies with small sample sizes and low doses of supplemental choline. Hence, the current study aims to re-establish the effects of high levels of supplemental choline chloride on weight gain, feed intake and feed efficiency and carcass characteristics for the modern strains of commercial broiler.

MATERIALS AND METHODS

Experimental birds

240 day old, unsexed, commercial broiler chicks were purchased from the chicken hatchery for the study purpose. A broiler shed (300 sft) was prepared. The floor of the shed was subdivided into twelve compartments and birds were randomly allocated there. Birds were brooded under single-tired electric brooder at 95 ˚F, 90 ˚F, 85 ˚F and 80 ˚F for the 1st, 2nd, 3rd and 4th week respectively. Birds were reared on rice husk litter. Room temperature and humidity was maintained using 200 watt incandescent lamps and exhaust fans.

Diets

Experimental diets were dry mash type (Table 1). Birds had unrestricted access to feed and water by plastic hanging feeders and bell type drinkers, except when they were starved for 4 h before slaughter. All diets were prepared with maize, rice polish, soybean oil, soybean meal, protein concentrate and other trace nutrients (NRC, 1994). Choline chloride was tested by non aqueous titration method. Concentration of choline in choline chloride was 58.3 g / 100 g. The standard premix was mixed with a small quantity of rice polish before incorporation into the experimental diets. All diets were analyzed in triplicate for moisture, ME, CP, CF, EE, Ca, P, lysine and methionine as per AOAC (1990). All birds were supplemented iso caloric and iso nitrogenous diets. Choline chloride was supplemented at 0 g / 100 g, 0.1 g / 100 g, 0.2 g / 100 g and 0.3 g / 100 g for T_0, T_1, T_2 and T_3 treatment groups respectively. Nutrient density in the experimental diet was maintained as per Singh (1980).

Design of the experiment

The experiment was carried out following completely randomized design (Gomez and Gomez, 1984; Winer *et al.* 1991). Birds were weighed randomly and divided into four treatment groups designated as T_0, T_1, T_2 and T_3. Each treatment was divided into three replicates having 20 birds per replicate.

Dressing the carcass

Feed and water were withdrawn 4 h before slaughter. From all replicates, a total 192 birds were slaughtered at 4th and 5th weeks of age by cervical dislocation. Carcass weights were recorded after removal of feather, feet, head and viscera. Hot carcass weight was recorded immediately after evisceration (Jones, 1984).

Abdominal fat, consisting of fat surrounding the gizzard, proven triculus and in the abdominal cavity was removed and weighed immediately. The weight of heart and liver were recorded.

Statistical analysis

Individual pens of the birds were treated as experimental unit. Data related to weight gain, feed intake and FCR were analyzed using ANOVA by using Stata (2009) and SPSS (2007). Correlation co-efficient matrix was calculated using SPSS 16.0. Means showing significant differences were compared by Duncan's New Multiple Range Test (Duncan, 1955). Statistical significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSION

Live weight gain

From 1st to 4th weeks, weight gain did not differ ($P>0.05$) irrespective of the level of choline supplementations (Table 2). However, unlike the 1st to 4th weeks, rate of gain differed ($P<0.05$) at 5th week of age. Mean values for weight gain at the 5th week were 67.8, 68.6, 72.4 and 68.7 g/bird/d for T_0, T_1, T_2 and T_3 treatment groups respectively. At 5th week, weight gain was maximum (72.4 g/bird/d) in birds fed diet supplemented with 0.2 g / 100 g choline chloride (T_2) and minimum (67.8 g/bird/d) in birds fed diet supplemented without choline chloride (T_0). It was evident that supplementation of choline substantially increased weight gain in broiler. The result is in agreement with Pesti *et al.* (1980) that supplemented choline in absence of methionine and obtained similar results. However, this finding is contradictory with other researchers (Swain and Johri 2000; Tsiagbe *et al*. 1987) who did not find any effect of choline supplementation on weight gain. Rama Rao *et al*. (2001) reported that while level of supplemental choline chloride increased from 0-1520 mg/kg the efficiency of ME (MJ/egg) and CP (g/egg) improved from 2.6 to 2.4 and 41.8 to 39.2 in broiler breeder. In another study, Emmert and Baker (1997) used a choline-deficient basal diet and found an almost linear response to the incremental supplementation of choline chloride up to 1115 mg/kg feed in chicks from 10-22 days of age, which indicates the positive role of supplemental choline in weight gain of birds. However, in the same study, increasing choline chloride up to 2000 mg/kg resulted in further weight gain to a lesser extent and levels in excess of this had no benefit. The effects of choline chloride over supplementary betaine in chicks have also been studied (Emmert and Baker, 1997). A choline free basal diet was used. Adding choline chloride had an almost linear effect on weight gain.

Table 1 Composition of the rations

Ingredient (g/100g)	T_0		T_1		T_2		T_3	
	Starter	Finisher	Starter	Finisher	Starter	Finisher	Starter	Finisher
Maize	57.4	62	57.4	62	57.4	62	57.4	62
Rice polish	4.9	3.7	4.8	3.6	4.7	3.5	4.6	3.4
Soybean oil	1.9	3.5	1.9	3.5	1.9	3.5	1.9	3.5
Soybean meal	26	21.5	26	21.5	26	21.5	26	21.5
Protein concentrate	7.5	7	7.5	7	7.5	7	7.5	7
Lime stone	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Dicalcium phosphate	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
DL-Methionine	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cholene chloride	-	-	0.1	0.1	0.2	0.2	0.3	0.3
Vitamin B premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin A, D, E and K	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Feedzyme	0	0	0	0	0	0	0	0
Common salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100	100
Analytical value								
Metabolizable energy (kcal/kg)	3003	3153	3000	3150	3004	3155	3001	3152
Crude protein (g/100g)	22.1	20.0	22.1	20.0	22.1	20.0	22.1	20.0
Calcium (g/100g)	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Phosphors (g/100g)	0.8	0.7	0.8	0.7	0.8	0.7	0.8	0.7
Avail phos (g/100g)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Lysine (g/100g)	1.4	1.3	1.4	1.3	1.4	1.3	1.4	1.3
Methionine (g/100g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Cyst + Meth (g/100g)	0.9	0.8	0.9	0.8	0.9	0.8	0.9	0.8
Tryptophan (g/100g)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Crude fibre (g/100g)	3.3	3.0	3.3	3.0	3.3	3.0	3.3	3.0
Ether extract (g/100g)	5.3	6.8	5.3	6.8	5.3	6.8	5.3	6.8

T_0: diet without choline chloride; T_1: diet containing 0.1 g / 100 g choline chloride; T_2: diet containing 0.2 g / 100 g choline chloride; T_3: diet containing 0.3 g / 100 g choline chloride.

Table 2 Effect of supplementing choline on live weight gain (g/bird/day) in commercial broiler (n=240)

Weight gain (g/bird)	Dietary treatment groups				SE	Significant
	T_0	T_1	T_2	T_3		
Weight gain at 1st wk	16.2±0.71	16.5±0.96	17.0±1.05	16.5±0.43	0.62	NS
Weight gain at 2nd wk	43.6±0.73	44.0±1.13	44.9±2.22	43.9±1.59	1.17	NS
Weight gain at 3rd wk	58.7±2.10	58.9±1.13	60.2±2.10	58.9±1.10	1.24	NS
Weight gain at 4th wk	66.0±1.01	67.1±1.05	68.4±1.09	67.8±1.02	0.69	NS
Weight gain at 5th wk	67.8^b±1.01	68.6^b±1.21	72.4^a±1.39	68.7^b±1.28	0.76	*

SE: standard error and NS: non significant.

* (P<0.05).

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

The addition of betaine at a concentration of 500 mg/kg feed to the basal diet and to diets containing approximately 570 mg choline chloride/kg feed had no effect on performance of bird. It indicates that the essential requirement for choline must be met before responses to betaine can be expected.

Feed intake

No variations (P>0.05) in feed intake were evident from 1st to 4th weeks irrespective of the level of choline supplementations in all dietary treatment groups (Table 3). Unlike that, intake differed markedly (P<0.05) at 5th week of age. Mean values for feed intake at the 5th week were 169.9, 168.2, 165.7 and 168.4 g/bird/d for T_0, T_1, T_2 and T_3 treatment groups respectively (Table 3). At the 5th week, feed intake was maximum (169.9 g/bird) in birds fed diet without choline chloride (T_0) and minimum (165.7 g/bird) in birds fed diet supplemented with 0.2 g / 100g choline chloride (T_2).

Linear decrease of feed intake with increasing levels of supplemental choline could be due to the reason that increasing levels of choline supplementation improved the efficiency of utilization of feed leading to gradual decrease in feed intake. This result is consistent with Ohta and Ishibashi (1995). However, in contrast to present study, Tsiagbe *et al.* (1987) reported lower feed intake fed basal diet. However, Swain and Johri (2000) did not find any effect of supplemental choline on feed consumption.

Feed conversion ratio (FCR)

Feed conversion ratio didn't differ (P>0.05) for the 1st and 2nd weeks despite level of choline supplementations in all dietary treatment groups (Table 4). However, it differed (P<0.05) at the 3rd, 4th and 5th weeks of age. FCR was most superior (1.72) in birds fed diet supplemented with 0.2 g / 100g choline chloride (T_2). Unlike T_2 comparatively poor FCR (1.84) was found in birds fed diet without choline chloride (T_0). Supplementation of choline chloride substantially improved feed efficiency in broiler from 1.84 to 1.72. The result is consistent with Baker *et al.* (1999) who found significant response in maize soybean based diet supplemented with choline chloride. Emmert and Baker (1997) used a choline free basal diet and obtained almost linear effect on feed efficiency. Response of commercial broiler to different dietary choline chloride supplementation (0, 400, 800, 1600 mg/kg) was measured with typical corn / soybean meal based diets (INRA, 1997). Feed conversion was improved from 1.71 to 1.66 kg. In another study, Swain and Johri (2000) supplemented methionine and choline either individually or in combinations but did not find any improvement in the efficiency of food utilization of broilers at 42 d. However, chicks fed on diets supplemented with 2000 mg/kg choline exhibited numerical improvement in the values of food conversion efficiencies. Similar results were obtained by Blair *et al.* (1986) and Tsiagbe *et al.* (1987).

Relationship between performance and carcass parameters

Weight gain and feed intake were positively correlated on 4th and 5th weeks of age with all the carcass parameters (Table 5 and 6). It clearly indicated that as either weight gain or feed intake increased progressively, the weight of shank, drumstick bone, thigh bone, dressed carcass, breast meat, thigh meat, drumstick meat, skin, wing meat, heart and lung also increased simultaneously in the same fashion. Numerically, FCR had a negative relationship with weight gain and feed intake. However, biologically it reveals that increase in weight gain or feed intake improved FCR. From correlation coefficient matrix (Table 7 and 8) it was evident that all carcass parameters were positively correlated among themselves.

Table 3 Effect of supplementing choline chloride on feed intake (g/bird/day) of commercial broiler (n=240)

Feed intake (g/bird)	Dietary treatment groups				SE	Significant
	T_0	T_1	T_2	T_3		
Feed intake at 1st wk	23.8±2.11	23.6±1.10	22.9±1.02	22.6±1.95	0.60	NS
Feed intake at 2nd wk	57.3±2.10	56.7±2.0	55.3±1.02	57.3±1.90	0.72	NS
Feed intake at 3rd wk	96.9±2.02	95.7±2.01	91.2^b±2.10	95.7±2.01	1.26	NS
Feed intake at 4th wk	127.8±2.2	127.8±2.10	123.9±1.54	125.9±1.56	0.93	NS
Feed intake at 5th wk	169.9^a ±0.9	168.2^a±0.87	165.7^b±0.73	168.4^a±0.94	0.66	*

SE: standard error and NS: non significant.
* (P<0.05).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 4 Effect of supplementing choline chloride on FCR of commercial broiler (n=240)

FCR	Dietary treatment groups				SE	Significant
	T_0	T_1	T_2	T_3		
FCR at 1st wk	1.08±0.02	1.06±0.05	1.01±0 .04	1.02±0.03	0.01	NS
FCR at 2nd wk	1.24±0.02	1.21±0.02	1.16±0.02	1.21±0.03	0.01	NS
FCR at 3rd wk	1.43^a±0.03	1.41^a±0.03	1.33^b±0.01	1.40^a±0.03	0.01	*
FCR at 4th wk	1.61^a±0.02	1.58^a±0.05	1.49^b±0.02	1.56^a±0.02	0.01	*
FCR at 5th wk	1.84^a±0.07	1.81^a±0.08	1.72^b±0.09	1.80^a±0.06	0.01	**

SE: standard error and NS: non significant.
FCR: feed conversion ratio.
* (P<0.05) and ** (P<0.01).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 5 Association between carcass parameter and productive performance of broiler at 4th week (n=192)

Parameter	Shank (wt)	Drum bone (wt)	Thigh bone (wt)	Dressed (wt)	Breast meat (wt)	Thigh meat (wt)	Drum meat (wt)	Skin (wt)	Wing meat (wt)	Heart (wt)	Lung (wt)
Weight gain (g/bird/day)	0.37	0.65*	0.44	0.75**	0.29	0.63*	0.29	0.420	0.79**	0.195	0.67*
Feed intake (g/bird/day)	0.38	0.84**	0.73**	0.80**	0.69*	0.72**	0.59*	0.389	0.88**	0.010	0.49
FCR	-0.38	-0.82**	-0.72**	-0.79**	-0.68*	-0.71**	-0.59*	-0.427	-0.86**	-0.004	-0.47

wt: weight.
* (P<0.05) and ** (P<0.01).
FCR: feed conversion ratio.

Table 6 Association between carcass parameter and productive performance of broiler at 5th week

Parameter	Shank (wt)	Drum. bone (wt)	Thigh bone (wt)	Dressed (wt)	Breast meat (wt)	Thigh meat (wt)	Drum meat (wt)	Skin (wt)	Wing meat (wt)	Heart (wt)	Lung (wt)
Weight gain (g/bird/day)	0.35	0.82**	0.69*	0.76**	0.67*	0.69*	0.57	0.43	0.85**	0.03	0.46
Feed intake (g/bird/day)	0.34	0.76**	0.66*	0.74**	0.62*	0.64*	0.51	0.49	0.81**	0.04	0.41
FCR	-0.36	-0.83**	-0.67*	-0.75**	-0.67*	-0.68*	-0.59*	-0.42	-0.85**	-0.03	-0.46

wt: weight.
* (P<0.05) and ** (P<0.01).
FCR: feed conversion ratio.

Table 7 Correlation coefficient matrix of carcass parameter at 4th week (n=192)

Parameter	Shank (wt)	Drum bone (wt)	Thigh bone (wt)	Dressed (wt)	Breast meat (wt)	Thigh meat (wt)	Drum meat (wt)	Skin (wt)	Wing meat (wt)	Heart (wt)	Lung (wt)
Shank (wt)	1	-	-	-	-	-	-	-	-	-	-
Drum stick (wt)	0.32	1	-	-	-	-	-	-	-	-	-
Thigh bone (wt)	0.33	0.39	1	-	-	-	-	-	-	-	-
Dressed (wt)	0.42	0.56	0.82**	1	-	-	-	-	-	-	-
Breast meat (wt)	0.55	0.59*	0.67*	0.42	1	-	-	-	-	-	-
Thigh meat (wt)	0.19	0.62*	0.700*	0.770**	0.577*	1	-	-	-	-	-
Drum meat (wt)	0.54	0.61*	0.35	0.28	0.767**	0.36	1	-	-	-	-
Skin (wt)	0.19	0.00	0.32	0.39	0.18	0.26	0.14	1	-	-	-
Wing meat (wt)	0.47	0.72**	0.72**	0.868**	0.57	0.71**	0.601*	0.51	1	-	-
Heart (wt)	0.05	0.19	0.07	0.22	0.23	0.09	0.11	0.08	0.02	1	-
Lung (wt)	0.04	0.43	0.42	0.612*	0.18	0.68*	0.34	0.42	0.76**	0.06	1

wt: weight.
* (P<0.05) and ** (P<0.01).

Table 8 Correlation coefficient matrix of carcass parameter at 5th week (n=192)

Parameter	Shank (wt)	Drum bone (wt)	Thigh bone (wt)	Dressed (wt)	Breast meat (wt)	Thigh meat (wt)	Drum meat (wt)	Skin (wt)	Wing meat (wt)	Heart (wt)	Lung (wt)
Shank (wt)	1	-	-	-	-	-	-	-	-	-	-
Drum stick (wt)	0.31	1	-	-	-	-	-	-	-	-	-
Thigh bone (wt)	0.33	0.39	1	-	-	-	-	-	-	-	-
Dressed (wt)	0.41	0.56	0.82**	1	-	-	-	-	-	-	-
Breast meat (wt)	0.54	0.58*	0.67*	0.42	1	-	-	-	-	-	-
Thigh meat (wt)	0.19	0.61*	0.70*	0.77**	0.57*	1	-	-	-	-	-
Drum meat (wt)	0.54	0.61*	0.36	0.27	0.76**	0.36	1	-	-	-	-
Skin (wt)	0.19	0.00	0.32	0.39	0.18	0.26	0.14	1	-	-	-
Wing meat (wt)	0.47	0.71**	0.72**	0.86**	0.57	0.71**	0.60*	0.51	1	-	-
Heart (wt)	0.05	0.19	0.07	0.22	0.23	0.09	0.11	0.08	0.02	1	-
Lung (wt)	0.04	0.43	0.42	0.613*	0.18	0.68*	0.34	0.42	0.76**	0.06	1

wt: weight.
* (P<0.05) and ** (P<0.01).

It indicated that either positive or negative change in any component of the carcass exhibited same effect in other parts.

CONCLUSION

Higher density of metabolizable energy in diets stimulated weight gain, improved FCR and reduced marketing age of commercial broiler sustaining positive relationship among carcass parameters. It could therefore be concluded that choline chloride could be supplemented at a rate of 0.2 g / 100 g to improve carcass merit in commercial broiler.

ACKNOWLEDGEMENT

I acknowledge University Grants Commission (UGC), Bangladesh for financial support and Animal Nutrition Laboratory, Chittagong Veterinary and Animal Science University for technical support.

REFERENCES

AOAC. (1990). Official Methods of Analysis. Vol. I. 15th Ed. Association of Official Analytical Chemists, Arlington, VA.

Artom C. (1953a). Lipid metabolism. *Ann. Rev. Biochem*. **22,** 211-232.

Artom C. (1953b). Role of choline in the oxidation of fatty acids by the liver. *J. Biol. Chem*. **205,** 101-111.

Artom C. (1953c). Role of choline in the oxidation of fatty acids by the isolated liver. *Nature*. **171,** 347-348.

Artom C. (1956). Effects of some dietary factors on the metabolism of fatty acids in liver preparations. *J. Biol. Chem*. **223,** 389-398.

Baker D.H., Edwards H.M., Strunk C.S., Emmert J.L., Peter C.M., Mavromichalis I. and Parr T.M. (1999). Single versus multiple deficiencies of methionine, zinc, riboflavin, vitamin B_6, and choline elicit surprising growth responses in young chicks. *J. Nutr*. **129,** 2239-2246.

Baker D.H., Halpin K.M., Czarnecki G. and Parsons C.M. (1983). The choline methionine interrelationship for growth of the chick. *Poult. Sci*. **62,** 133-137.

Blair M.E., Potter L.M., Bliss B.A. and Shelton J.R. (1986). Methionine, choline and sulfate supplementation of practical type diets for young turkeys. *Poult. Sci*. **65,** 130-137.

Duncan D.B. (1955). Multiple ranges and multiple ‘F’ test. *Biometrics*. **11,** 1-42.

Emmert J.L. and Baker D.H. (1997). A chick bioassay approach for determining the bioavailable choline concentration in normal and overheated soybean meal, canola meal and peanut meal. *J. Nutr*. **27,** 745-752.

Garrow T.A. (2007). Choline. Pp. 459-487 in Handbook of Vitamins. J. Zempleni, R.B. Rucker, D.B. McCormick and J.W. Suttie, Eds. Boca Raton (FL): CRC Press.

Gomez A.K. and Gomez A.A. (1984). Statistical Procedures for Agricultural Research. Willy and Sons. New York.

Griffith M., Olinde A.J., Schexnailder R., Davenport R.F. and Mcknight W.F. (1969). Effect of choline, methionine and vitamin B_{12} on liver fat, egg production and egg weight in hens. *Poult. Sci*. **48,** 2160-2172.

Hudgins L.C., Hellerstein M.K., Seidman C.E., Neese R.A., Tremaroli J.D. and Hirsch J. (2000). Relationship between carbohydrate-induced hypertriglyceridemia and fatty acid synthesis in lean and obese subjects. *J. Lipid Res*. **41,** 595-604.

INRA. (1997). Nutrition of laying hens. Pp. 214-219 in Feeding of Non-Ruminant Livestock. J. Wiseman, Ed. Butterworth and Co. Ltd., London.

Jones R. (1984). A Standard Method of Dissection of Poultry for Carcass Analysis. West of Scottland Agricultural College, Technical Bulletin No. 222. Ary. Scottland.

Jukes T.H. (1940). Effects of choline and other supplements on perosis. *J. Nutr*. **20,** 445-458.

NRC. (1994). Nutrient Requirements of Poultry. 9th Rev. Ed. National Academy Press, Washington, DC.

Ohta Y. and Ishibashi T. (1995). Effect of dietary glycine on reduced performance by deficient and excessive methionine in broilers. *Jpn. Poult. Sci*. **32,** 81-89.

Pearce J. (1975). The effects of choline and inositol on hepatic lipid metabolism and the incidence of the fatty liver and kidney syndrome in broilers. *Br. Poult. Sci*. **16,** 565-570.

Pesti G.M., Harper A.E. and Sunde M.L. (1980). Choline / methionine nutrition of starting broiler chicks. Three models for estimating the choline requirement with economic considerations. *Poult. Sci*. **59,** 1073-1081.

Rama Rao S.V., Sunder G.S., Reddy M.R., Praharaj N.K., Raju M.V. and Panda A.K. (2001). Effect of supplementary choline on the performance of broiler breeders fed on different energy sources. *Br. Poult. Sci*. **42,** 362-367.

Scott M.L., Nesheim M.C. and Young R.J. (1982). Nutrition of the Chicken. Publishers, Ithaca, New York.

Singh R.A. (1980). Poultry Production. Ramanath Mazumder Street, Ballygunj, Kolkata. Kalyani Publishers, India.

SPSS. (2007). SPSS for Windows, Version 16.0. Chicago, SPSS Inc.

Stata. (2009). Stata Statistical Software. Version 11. TX: StataCorp LP., College Station, USA.

Swain B.K. and Johri T.S. (2000). Effect of supplemental methionine, choline and their combinations on the performance and immune response of broilers. *Br. Poult. Sci*. **41,** 83-88.

Tsiagbe V.K., Cook M.E., Harper A.E. and Sunde M.L. (1987). Efficacy of cysteine in replacing methionine in the immune responses of broiler chicks. *Poult. Sci*. **66,** 1138-1146.

Whitehead C.C. and Randall C.J. (1982). Interrelationships between biotin, choline and other Bvitamins and the occurrence of fatty liver and kidney syndrome and sudden death syndrome in broiler chickens. *Br. J. Nutr*. **48,** 177-184.

Winer B.J., Brown R. and Michels K.M. (1991). Statistical Principles in Experimental Design. McGraw-Hill, New York.