Indian Journal of Poultry Science (2024) 59(3): 271–280, Research Article
https://doi.org./https://doi.org/10.56093/ijps.v59i3.06

Effect of dietary supplementation and in ovo feeding of alpha-ketoglutaric acid on the growth performance, development of lymphoid and digestive organs and carcass quality traits of broilers

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(Received on December 12, 2023; accepted for publication on August 09, 2024)

ABSTRACT

Kherde, A.R., Bhattacharyya, A., Shukla, P.K., Bondar, B.B., Patel, M., Gulhane, P.S. and Sharma, M. 2024. Effect of dietary supplementation and in ovo feeding of alpha-ketoglutaric acid on the growth performance, development of lymphoid and digestive organs and carcass quality traits of broilers. Indian Journal of Poultry Science, 59(3): 271-280.

The present study was conducted to assess the effect of dietary supplementation vis-à-vis in ovo feeding of alpha-ketoglutaric acid (AKG) on the performance of commercial broilers. A total of 375 fertile eggs of CARI-VISHAL birds were set for incubation for first 18 days in the setter and last 3 days in the hatcher. On the 18d, *in ovo* feeding was done at the broad end of the egg. The eggs were divided into three groups: un-injected control, sham control and *in ovo* injected with AKG (0.6 ml of 1.5% AKG solution). After hatching, 180 day-old chicks were reared for feeding trials. Chicks from these three groups were further divided into two subgroups, each with three replicates and ten chicks per replicate. One subgroup received a basal diet, while the other received a basal diet with 1% AKG supplementation diet. Birds were reared for 42 days (6 weeks) and kept on a basal or control diet [BIS, 2007; broiler starter diet till 3 weeks and thereafter broiler finisher diet till 6 weeks]. Day-old and 1st week body weight of *in ovo* AKG group birds were significantly higher and weekly body weight of birds from 2nd week onwards was significantly higher ($p \leq 0.01$) till the end of the experiment after dietary supplementation of AKG as compared to basal diet group birds. During 2nd and 3rd week of age, body weight gain of dietary AKG supplemented group was significantly higher ($p=0.001$) than the basal diet group. Small intestine weight was significantly lower ($p \leq 0.01$) in basal diet supplemented with AKG than basal diet. However, large intestine length was significantly higher ($p=0.005$) in the in ovo AKG group than un-injected control. Thus, it may be concluded that *in ovo* feeding of AKG and/or dietary supplementation of AKG did not result in any significant difference in weekly body weight gain, weekly feed consumption, weekly feed conversion ratio, carcass quality traits and development of digestive and lymphoid organs. However, body weight of day-old and 1st week age chicks was significantly higher after *in ovo* feeding of AKG as compared to basal diet.

Keywords: *In ovo* feeding, AKG, Glutamin, Growth, Carcass quality, Broiler

INTRODUCTION

India is the third largest producer of eggs and ranks sixth in poultry meat production in the world (BAHS, 2022). Protein is an essential constituent of all tissues of animal body and has major effect on growth performance of the bird (Kamran, 2004). Dietary protein is often the most expensive component of broiler diets. Reducing dietary protein levels and using synthetic amino acids reduce feed costs. Feeding high amino acid density diets improve feed conversion and increase weight gain and breast meat yield of broiler chickens (Kidd *et al.*, 2004).

Alpha-ketoglutaric acid (AKG) is a central molecule in the tricarboxylic acid cycle (Krebs cycle). AKG can be synthesized when glucose or oxaloacetate is combined with pyruvate, giving it another synonym as 2-oxoglutarate and 2-ketoglutarate (Harrison and Pierzynowski, 2008). It can be rapidly transaminated, by glutamate (Glu) dehydrogenase, to glutamic acid and then further aminated by glutamine (Gln) synthetase to form Gln (Prandini *et al.*, 2012; Wang *et al.*, 2016). AKG is a precursor of amino acid glutamine and is known to provide nitrogen and an energy source for the proliferation of immune and intestinal mucosal cells and is required together with cysteine to synthesize antioxidants such as glutathione (Newsholme, 2001; Obled, 2003). AKG has various physiological functions, including acting as an antioxidant (Lemire *et al.*, 2010; Long and Halliwell, 2011) or as an anticancer agent (Tennant and Gottlieb, 2010; Rzeski *et al.*, 2012) and enhancing host-defense (Muhling *et al.*, 2010). Ammonia is toxic to the body; however, AKG exerts a beneficial effect on nitrogen metabolism by facilitating ammonium conversion to amino acids and protein and by reducing levels of ammonium in the body (Wiren *et al.*, 2002).

The development of chicken embryos completely relies on the nutrients present in the egg. The first administration of *in ovo* delivery of exogenous material was reported in the 1980s for vaccination against Marek's disease (Sharma and Burmester, 1982). *In ovo* feeding involves the injection of exogenous nutrients into hatching eggs, which can promote the growth and development of poultry during incubation and after hatching (Daraji *et*

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al., 2012; Bhanja and Mandal, 2005; Coskun*et al*., 2017; Ohta and Kidd, 2001; Salmanzadeh, 2012; Uni and Ferket, 2004).

Comprehensive studies on the effect of dietary supplementation of AKG on the performance of broilers are limited. Further, studies on *in ovo* feeding of AKG in broilers are also limited as studies have taken place only on the growth performance. Further, literature pertaining to dietary supplementation vis-à-vis *in ovo* feeding of AKG in commercial broilers is scanty. Hence, the present study was carried out to study the effect of dietary supplementation of alpha-ketoglutaric acid vis-a-vis *in ovo* feeding of alpha-ketoglutaric acid on performance of commercial broilers.

MATERIALS AND METHODS

Eggs

A total of 375 fertile eggs of CARI-VISHAL birds were purchased from ICAR Central Avian Research Institute, Izatnagar, Bareilly. These eggs were set for incubation for first 18 days in the setter and the last 3 days in the hatcher. On the 18th day, eggs were candled and dead-in-shell eggs were discarded. Further, the remaining fertile eggs were divided into three treatment groups: un-injected control, sham control and *in ovo* injected with alpha-ketoglutaric acid.

Preparation of solution of alpha-ketoglutaric acid for in ovo feeding

Alpha-ketoglutaric acid was procured from SRL Pvt. Ltd. Alpha-ketoglutaric acid (AKG) is a white crystalline powder, which is soluble in water. 1.5 % alpha-ketoglutaric acid solution was prepared by adding 1.5 g of alpha-ketoglutaric acid in 100 ml of double distilled water and further, it was used for *in ovo* injection. The solution was warmed to a temperature of 30°C before injecting.

In ovo feeding

In ovo feeding was done on 18th day of incubation. Adherence to the prescribed biosafety and good laboratory practice protocols were diligently maintained throughout the *in ovo* feeding process.

Experimental design

Birds were reared for 42 days (6 weeks) and kept on a basal or control diet [BIS, 2007; broiler starter diet till 3 weeks and thereafter broiler finisher diet till 6 weeks]. The experimental design was 3×2 CRD.

Treatment	*In ovo* Injection	Diet
T1	Un-injected (Control)	Basal Diet
T2	Un-injected (Control)	Basal Diet + supplementation of 1% AKG
T3	Injected with 0.6 ml of distilled water / egg (Sham Control)	Basal Diet
T4	Injected with 0.6 ml of 1.5% AKG solution /egg (Sham Control)	Basal Diet +supplementation of 1% AKG
T5	Injected with 0.6 ml of distilled water/egg	Basal Diet
T6	Injected with 0.6 ml of 1.5% AKG solution/egg	Basal Diet + supplementation of 1% AKG

Growth performance parameters

Weekly body weight, group feed consumption and mortality was recorded. Feed conversion ratio (feed intake: body weight gain) of 0 to 6 weeks were calculated at the end of the experiment

Development of lymphoid and digestive organs and carcass quality traits

At the time of slaughter, 2 male and 2 female birds from each group i.e. total 24 birds were taken after 6 weeks of age for studying various slaughter traits *viz*. pre-slaughter fasting shrinkage in live weight (%), debleeding weight (%), defeathered weight (%), dressed weight (%), yield after evisceration loss or ready to cook yield (%), giblets yield (%), ready-to-cook yield (%) and development of digestive organs. Further, the percent yield of cut-up-parts (thighs, drumsticks, breast, back, neck and wing) on eviscerated carcass yield was determined.

The birds were starved for 12 hours before slaughter. However, drinking water was provided *ad lib*. During the starvation period, their body weights were recorded after starvation. The birds were sacrificed by improved Kosher method, bled for 1.5 to 2 minutes and defeathered. The birds were dressed by cutting the head at atlanto-occipital joint, leg at hock joint and oil gland located at the base of the tail and weighed.

Evisceration was done by making a slit opening at the neck skin to remove oesophagus and trachea, vertical cut below the tip of breast bone to remove viscera. Heart, liver and gizzard were separated and cleaned. The internal lining of gizzard and pericardium of heart were removed before weighing them.

Further, the length and weight of different digestive organs (proventriculus, small intestine, large intestine and caecum) were measured separately at sixth week of age.

Statistical Analysis

The data pertaining to various parameters were analyzed statistically as per the standard procedure (Snedecor and Cochran, 1989) and difference between the treatment means were obtained by using Duncan multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Weekly body weight

There were no significant differences in average weekly body weight among the treatment groups after *in ovo* feeding except at day-old, 1st week and 2nd week of age (Table 1). Day-old and 1st week body weight of *in ovo* AKG group birds were significantly higher (p≤0.04, p≤0.02) than un-injected control at day old and sham control at 1st week of age, respectively. Further, 2nd week body weight of un-injected control group birds was significantly higher (p≤0.01) than sham control. This is in agreement with the result of other studies (Salmanzadeh *et al*., 2016; Rahardja *et al*., 2018) who reported that weight of newly hatched chicks was significantly higher after *in ovo* feeding of glutamine than birds in control and sham groups. In both these studies, *in ovo* feeding of glutamine was done to provide amino acid for gluconeogenesis, reduce albumin catabolism in late-term embryo as neonatal chicken depend on gluconeogenesis from amino acids thereby resulting in depletion of muscle protein reserves and reduction of hatching weight (Bhattacharyya *et al*., 2018). Thus, *in ovo* feeding of amino acid glutamine involved sparing amino acid for muscle and tissue protein synthesis and consecutively improving body weight at hatch. In the present study, AKG, precursor of glutamine was *in ovo* injected. However, the beneficial effect of higher body of chicks of *in ovo* fed group was not continued from 2nd week onwards. It is still unclear whether AKG's effect on broiler performance is through glutamine metabolism (Gupta *et al*., 2022). In a study, Gupta *et al*. (2022) noted that a significant quadratic increase in body weight was observed for body weight on day 7 and day 21 after increasing the AKG dose of *in ovo* feeding to 1.5% AKG dissolved in distilled water.

It was observed that after dietary supplementation of AKG, average weekly body weight of birds during 2nd, 3rd, 4th, 5th and 6th week of age was significantly higher (p=0.002, p=0.001, p=0.002, p=0.02, p=0.03) than basal diet group. However, there were no significant differences observed in day old and 1st week body weight of birds after dietary supplementation of AKG. Similarly, Soltan (2009) reported that 1% glutamine (Gln) supplementation significantly (p≤0.05) improved body weight in broiler chickens. In contrary to these studies, Sakamoto *et al*. (2006) and Maiorka *et al*. (2000) did not observe any significant effect on body weight after dietary glutamine supplementation. This discrepancy may be due to the strain/ line of broilers, mode of supplementation and/or compound employed.

Table 1: Effect of dietary supplementation and *in ovo* feeding of alpha-ketoglutaric acid on average weekly body weight (g) of commercial broilers during 0-6 weeks of age

Group	Age in weeks						
	Day-old	1st	2nd	3rd	4th	5th	6th
Un-injected + Basal diet	38.13	104.37	273.62	583.57	826.33	1141.57	1521.06
Un-injected + AKG supplementation	37.00	107.76	277.77	604.10	850.93	1173.96	1544.24
Sham control + Basal diet	38.10	97.76	243.51	527.99	774.24	1072.73	1462.23
Sham control + AKG supplementation	38.07	103.57	270.22	601.33	859.19	1169.85	1564.07
In ovo AKG + Basal diet	39.87	110.67	257.18	556.04	807.33	1125.10	1494.69
In ovo AKG + AKG supplementation	38.60	112.80	280.36	600.52	855.56	1146.22	1547.04
Treatment							
Un-injected	37.57^{a}	106.06^{ab}	275.69^{b}	593.84	838.63	1157.76	1532.65
Sham control	38.08^{ab}	100.67^{a}	256.87^{a}	564.66	816.71	1121.29	1513.15
In ovo AKG	39.23^{b}	111.74^{b}	268.77^{ab}	578.28	831.44	1135.66	1520.86
Diet							
Basal diet	38.70	104.27	258.10^{a}	555.87^{a}	802.64^{a}	1113.13^{a}	1492.66^{a}
AKG supplementation	37.89	108.04	276.12^{b}	601.99^{b}	855.22^{b}	1163.34^{b}	1551.79^{b}
Pooled SEM	0.29	1.60	3.65	7.99	9.24	11.21	12.98
Significance Level							
Treatment	p=0.04	p= 0.02	p=0.01	NS	NS	NS	NS
Diet	NS	NS	p=0.002	p=0.001	p=0.002	p=0.02	p=0.03
Treatment × Diet	NS	NS	NS	NS	NS	NS	NS

Means bearing different superscripts within a column differ significantly (p≤0.05)

NS: Not Significant (p≥0.05) SEM: Standard Error of Mean

There were no significant differences in interaction of dietary supplementation and *in ovo* feeding of AKG on average weekly body weight of birds

Weekly body weight gain

Result indicated that no significant differences were observed in average weekly body weight gain of birds after *in ovo* feeding throughout the experimental period except during 1st and 2nd week of age (Table 2). Weekly body weight gain at 1st week was significantly higher (p=0.03) in birds of *in ovo* AKG group than birds of sham control and during 2nd week, body weight gain of birds of un-injected control was significantly higher (p=0.01) than sham control and *in ovo* AKG group. However, Salmanzadeh *et al*. (2016) concluded that *in ovo* feeding of Gln showed higher weight gain (0–42 days of age) compared to chickens hatched from control and sham groups. Gupta *et al*. (2022) observed a quadratic increase in average daily gain from 8 to 21 days of age with 1.5% AKG dissolved in distilled water. Bartell and Batal (2007) and Soltan (2009) observed that a high inclusion of dietary Gln (1.5, 2 and 4 %) could be toxic and resulted in body weight depression. In our study, though significantly higher body weight gain was recorded in the first week, the positive effect could not carried over in the subsequent weeks. The plausible reason for this discrepancy has already been explained in the section weekly body weight.

There were no significant differences in weekly body weight gain after dietary supplementation of AKG, except 2nd and 3rd week body weight gain. Body weight gain during 2nd and 3rd week was significantly higher (p=0.001, p=0.001) in AKG supplementation group as compared to the basal diet group. Similarly, Bartell and Batal (2007) also observed that weight gain in 21 day old broiler chickens fed a diet supplemented with 1% Gln was significantly higher (11 % average improvement) in comparison with chickens fed without Gln. However, contrary to these results, Guo *et al*. (2017) concluded AKG supplementation did not significantly affect the growth performance of Cherry Valley ducks. In another study, Tomaszewska *et al*. (2020) reported dietary AKG supplementation did not influence body weight gain of laying hens. This discrepancy in results may be due to the varying levels of dietary supplementation of AKG and the type of birds subjected to dietary supplementation.

Interaction of *in ovo* feeding and dietary supplementation of AKG resulted no significant differences in average weekly body weight gain during 0-6 weeks of age except at 2nd week. This implies that *in ovo* feeding along with dietary supplementation of AKG did not impart any additional beneficial effect on body weight gain of broilers.

Weekly feed consumption

Result indicated that after *in ovo* feeding of AKG, no significant differences were observed in the average weekly feed consumption of birds throughout the experimental period except at 1st week of age (Table 3). At 1st week of age, average weekly feed consumption of birds of *in ovo* AKG fed group was significantly lower ($p \leq 0.05$) than birds of sham control and un-injected

Table 2: Effect of dietary supplementation and *in ovo* feeding of alpha-ketoglutaric acid on average weekly body weight gain (g) of commercial broilers during 0-6 weeks of age

Group	Age in weeks					
	1st	2nd	3rd	4th	5th	6th
Un-injected + Basal diet	66.23	169.25^{b}	309.95	242.76	315.24	379.49
Un-injected + AKG supplementation	70.76	170.01^{b}	326.33	246.83	323.02	370.29
Sham control + Basal diet	59.66	145.75^{a}	284.48	246.25	298.48	389.50
Sham control + AKG supplementation	65.50	166.65^{b}	331.11	257.85	310.67	394.22
In ovo AKG + Basal diet	70.81	146.50^{a}	298.87	251.29	317.76	369.59
In ovo AKG + AKG supplementation	74.20	167.56^{b}	320.16	255.04	290.67	400.81
Treatment						
Un-injected	68.50^{ab}	169.63^{b}	318.14	244.80	319.13	374.89
Sham control	62.58^{a}	156.20^{a}	307.80	252.05	304.57	391.86
In ovo AKG	72.50^{b}	157.03^{a}	309.51	253.16	304.21	385.20
Diet						
Basal diet	65.57	153.83^{a}	297.77^{a}	246.77	310.49	379.53
AKG supplementation	70.16	168.07^{b}	325.87^{b}	253.24	308.12	388.44
Pooled SEM	1.58	2.92	4.76	2.62	4.78	5.27
Significance Level						
Treatment	p=0.03	p=0.01	NS	NS	NS	NS
Diet	NS	p=0.001	p=0.001	NS	NS	NS
Treatment × Diet	NS	p=0.04	NS	NS	NS	NS

Means bearing different superscripts within a column differ significantly ($p \leq 0.05$)

NS: Not Significant ($p \geq 0.05$) SEM: Standard Error of Mean control group. In our present study, all *in ovo* AKG groups had numerically lower feed intake as compared to un-injected control. Similarly, Salmanzadeh *et al.* (2016) observed no significant difference in feed intake but numerically it was decreased with increasing concentration of glutamine throughout their experimental period. However, Gupta *et al.* (2022) reported quadratic increase in average daily feed intake with the concentration of AKG.

Results indicated that after dietary supplementation, no significant difference was observed in the average weekly feed consumption of birds during 0-6 week of age except at 4th and 5th week. Average weekly feed consumption of birds at 4th and 5th week was significantly higher (p≤0.05) in AKG supplementation group as compared to the basal diet group. Similarly, Murakami *et al.* (2007) observed no significant increase in feed intake in broilers fed diets containing vitamin E and 1 % dietary Gln. Further, Talpur *et al.* (2022) reported that there were no significant differences found in 0 to 42 days on average daily feed intake after dietary supplementation of AKG (1kg/t and 2kg/t). In addition, Tomaszewska *et al.* (2020) also reported that dietary supplementation with 1% AKG had no significant effect on the feed intake of laying hens.When interaction between dietary supplementation and *in ovo* feeding was considered, there were no significant differences in average weekly feed consumption.

Weekly feed conversion ratio

Results indicated that after *in ovo* feeding, there was no significant difference in the average weekly feed conversion ratio of birds during entire experimental period except at 1st week of age (Table 4). The average weekly feed conversion ratio during 1st week of age of birds in the *in ovo* AKG group was significantly better (P=0.001) than un-injected and sham control group. The results are in agreement with the observation of Gupta *et al.* (2022) who reported a quadratic increase in FCR (p=0.028) from days 0 to 7 with a higher concentration of *in ovo* AKG i.e. 1.5 % AKG. Similarly, Salmanzadeh *et al.* (2016) reported that *in ovo* feeding of glutamine improved feed conversion ratio throughout their experimental period in different phases.

There was no significant difference observed in weekly FCR after dietary supplementation of AKG. The data is in agreement with the observation of other studies (Soltan, 2009; Moghaddam and Ghamsari, 2013) who observed glutamine supplementation had no significant improvement in FCR as compared to the control group. Similarly, Guo *et al.* (2017) reported that AKG supplementation did not significantly affect the FCR of Cherry Valley ducks during their entire experimental period of 22 days.

When dietary supplementation along with *in ovo* feeding was taken into consideration, there was no significant difference in weekly FCR throughout the experimental period of 0-6 weeks of age of broilers.

Development of digestive and lymphoid organs

There were no significant differences observed in development of digestive (Table 5) and lymphoid organs (Table 6) after *in ovo* feeding and/or dietary

Table 3: Effect of dietary supplementation and *in ovo* feeding of alpha-ketoglutaric acid on average weekly feed consumption (g) of commercial broilers during 0-6 weeks of age

Group	Age in weeks					
	1st	2nd	3rd	4th	5th	6th
Un-injected + Basal diet	94.40	238.12	452.04	531.01	651.86	869.18
Un-injected + AKG supplementation	103.52	258.21	459.08	529.82	656.70	899.67
Sham control + Basal diet	97.47	211.98	471.60	514.84	615.03	873.09
Sham control + AKG supplementation	96.89	227.86	498.32	553.22	683.20	905.51
In ovo AKG + Basal diet	86.41	252.98	442.55	497.93	630.37	839.73
In ovo AKG + AKG supplementation	84.62	247.46	452.46	532.58	653.85	864.37
Treatment						
Un-injected	98.96[b]	248.17	455.56	530.42	654.28	884.42
Sham control	97.18[b]	219.92	484.96	534.03	649.12	889.30
In ovo AKG	85.51[a]	250.22	447.51	515.26	642.11	852.05
Diet						
Basal diet	92.76	234.36	455.40	514.60[a]	632.42[a]	860.67
AKG supplementation	95.01	244.51	469.95	538.54[b]	664.58[b]	889.85
Pooled SEM	2.40	6.73	7.44	6.13	8.05	8.34
Significance Level						
Treatment	p=0.05	NS	NS	NS	NS	NS
Diet	NS	NS	NS	p=0.05	p=0.05	NS
Treatment × Diet	NS	NS	NS	NS	NS	NS

Means bearing different superscripts within a column differ significantly (p≤0.05)

NS: Not Significant (p≥0.05) SEM: Standard Error of Mean

Table 4: Effect of dietary supplementation and *in ovo* feeding of alpha-ketoglutaric acid on weekly feed conversion ratio of commercial broilers during 0-6 weeks of age

Group	Age in weeks					
	1st	2nd	3rd	4th	5th	6th
Un-injected + Basal diet	1.43	1.41	1.46	2.19	2.07	2.29
Un-injected + AKG supplementation	1.47	1.52	1.41	2.15	2.03	2.44
Sham control + Basal diet	1.64	1.45	1.66	2.09	2.06	2.24
Sham control + AKG supplementation	1.48	1.36	1.51	2.15	2.21	2.30
In ovo AKG + Basal diet	1.22	1.73	1.49	1.98	1.99	2.28
In ovo AKG + AKG supplementation	1.14	1.48	1.42	2.09	2.26	2.16
Treatment						
Un-injected	1.45[b]	1.46	1.43	2.17	2.05	2.37
Sham control	1.56[b]	1.41	1.59	2.12	2.13	2.27
In ovo AKG	1.18[a]	1.60	1.45	2.04	2.13	2.22
Diet						
Basal diet	1.43	1.53	1.54	2.09	2.04	2.27
AKG supplementation	1.36	1.45	1.44	2.13	2.17	2.30
Pooled SEM	0.04	0.04	0.03	0.03	0.04	0.03
Significance Level						
Treatment	P=0.001	NS	NS	NS	NS	NS
Diet	NS	NS	NS	NS	NS	NS
Treatment × Diet	NS	NS	NS	NS	NS	NS

Means bearing different superscripts within a column differ significantly ($p \leq 0.05$)
NS: Not Significant ($p \geq 0.05$) SEM: Standard Error of Mean

Table 5: Effect of dietary supplementation and *in ovo* feeding of alpha-ketoglutaric acid on development of digestive organs of commercial broilers after 6 weeks of age

Group	Proventriculus (g/100g)	SI weight (g/100g)	LI weight (g/100g)	Caecal Wt. (g/100g)	SI length (cm/100g)	LI length (cm/100g)	Caecal length (cm/100g)
Un-injected + Basal diet	0.55	4.28	1.28	0.79	11.30	1.77	1.54
Un-injected + AKG supplementation	0.60	4.29	1.39	0.89	10.76	1.74	1.52
Sham control + Basal diet	0.55	5.06	1.50	0.91	12.36	1.70	1.63
Sham control + AKG supplementation	0.54	4.08	1.44	0.80	11.83	1.82	1.52
In ovo AKG + Basal diet	0.59	4.94	1.39	0.75	10.82	1.95	1.48
In ovo AKG + AKG supplementation	0.55	4.34	1.19	0.78	11.78	2.22	1.52
Treatment							
Un-injected	0.58	4.28	1.33	0.84	11.03	1.76[a]	1.53
Sham control	0.54	4.57	1.47	0.86	12.09	1.76[a]	1.57
In ovo AKG	0.57	4.64	1.29	0.76	11.30	2.08[b]	1.50
Diet							
Basal diet	0.56	4.76[b]	1.39	0.82	11.49	1.81	1.55
AKG supplementation	0.56	4.24[a]	1.34	0.82	11.45	1.93	1.52
Pooled SEM	0.02	0.11	0.04	0.03	0.23	0.05	0.03
Sig Level							
Treatment	NS	NS	NS	NS	NS	p=0.005	NS
Diet	NS	p=0.01	NS	NS	NS	NS	NS
Treatment × Diet	NS	NS	NS	NS	NS	NS	NS

Means bearing different superscripts within a column differ significantly ($p \leq 0.05$)
NS: Not Significant ($p \geq 0.05$) SEM: Standard Error of Mean : SI: Small intestine LI: Large intestine

supplementation of AKG except large intestine length and small intestine weight. Large intestine length of *in ovo* AKG group birds was significantly higher (p=0.005) as compared to un-injected control and sham control birds. Further, after dietary supplementation of AKG, the weight of small intestine was significantly lower (p≤0.01) in AKG supplementation as compared to basal diet group. Gupta *et al.* (2022) reported no significant differences among different treatment groups for the absolute and relative lengths of the small intestines and the average length of the cecum. Prabakar *et al.* (2023) concluded that supplementation of 0.5% glutamate increased intestinal length without affecting the other carcass characters in broiler chicken. Further, Chen *et al.* (2009) concluded that *in ovo* injection of glutamine and carbohydrates improves small intestine development in ducks. However, Barter and Batal (2007) observed an increase in the relative weight of duodenum and jejunum without any increase in thymus, spleen and bursa and in another experiment increase in relative weight of thymus and spleen of broilers fed diet containing 1% Gln in comparison with the birds fed a control diet. Thus, the contradictory results in the above studies suggest that further studies are needed before concluding the effect of AKG on the development of digestive and immune organs.

Carcass quality traits, yield of giblets and cut-up parts

There were no significant differences observed in carcass quality traits (Table 7), yield of giblets (Table 8) and cut-up-parts (Table 9) after *in ovo* feeding and/or dietary supplementation of AKG. Similarly, Mirnawati *et al.* (2019) also reported that glutamate up to 0.8 % did not affect the percentage of carcass. Lima *et al.* (2020) concluded that combination of L-glutamine and organic zinc in diets for broilers did not influence the carcass and cut yields in the carcass of birds at 21 days of age. Further, Dai *et al.* (2009) observed supplementation with 0.5 or 1 % Gln had no influence on breast absolute or relative weight at 42 days of age in broilers. The results of the present study clearly indicated that there was no adverse effect of AKG on the carcass quality characteristics, yield of giblets and cut up parts at 6 weeks of age.

CONCLUSION

It may be concluded that *in ovo* feeding of AKG and/or dietary supplementation of AKG did not result in any significant difference in weekly body weight gain, weekly feed consumption and weekly feed conversion ratio throughout the experimental period. However, body weight of day-old and 1st week age chicks was significantly higher after *in ovo* feeding of AKG as compared to basal diet. There was no significant difference in carcass quality traits, giblet yield, cut-up-parts and development of digestive and lymphoid organs among the different treatment groups.

ACKNOWLEDGEMENTS

The authors are thankful to the Dean, College of Veterinary Science and Animal Husbandry, Mathura, India and the Vice Chancellor of the University for providing necessary facilities to carry out the study.

Table 6: Effect of dietary supplementation and *in ovo* feeding of alpha-ketoglutaric acid on development of lymphoid organs of commercial broilers after 6 weeks of age (% live weight)

Group	Spleen weight (%)	Thymus weight(%)	Bursa weight(%)
Un-injected + Basal diet	0.15	0.43	0.34
Un-injected + AKG supplementation	0.17	0.41	0.31
Sham control + Basal diet	0.19	0.46	0.42
Sham control + AKG supplementation	0.16	0.37	0.31
In ovo AKG + Basal diet	0.16	0.41	0.33
In ovo AKG + AKG supplementation	0.13	0.53	0.32
Treatment			
Un-injected	0.16	0.42	0.32
Sham control	0.18	0.41	0.36
In ovo AKG	0.14	0.47	0.33
Diet			
Basal diet	0.17	0.43	0.36
AKG supplementation	0.15	0.44	0.31
Pooled SEM	0.01	0.03	0.02
Sig Level			
Treatment	NS	NS	NS
Diet	NS	NS	NS
Treatment × Diet	NS	NS	NS

NS: Not Significant (p≥0.05) SEM: Standard Error of Mean

Table 7: Effect of dietary supplementation and *in ovo* feeding of alpha-ketoglutaric acid on carcass quality traits of commercial broilers after 6 weeks of age (% live weight)

Group	Shrinkage (%)	Debleeding wt (%)	Defeathered wt (%)	Dressing wt (%)	Ready to cook yield(%)
Un-injected + Basal diet	7.78	96.32	79.83	71.53	53.69
Un-injected + AKG supplementation	9.32	96.20	79.99	70.70	52.69
Sham control + Basal diet	8.96	96.54	80.45	70.90	52.19
Sham control + AKG supplementation	11.45	96.61	80.45	71.44	53.29
In ovo AKG + Basal diet	9.01	96.57	79.92	70.99	51.14
In ovo AKG + AKG supplementation	8.16	96.63	80.27	71.59	53.59
Treatment					
Un-injected	8.55	96.26	79.91	71.12	53.19
Sham control	10.20	96.58	80.45	71.17	52.74
In ovo AKG	8.59	96.60	80.09	71.29	52.36
Diet					
Basal diet	8.58	96.47	80.07	71.14	52.34
AKG supplementation	9.64	96.48	80.24	71.24	53.19
Pooled SEM	0.44	0.10	0.24	0.21	0.47
Sig Level					
Treatment	NS	NS	NS	NS	NS
Diet	NS	NS	NS	NS	NS
Treatment × Diet	NS	NS	NS	NS	NS

NS: Not Significant ($p \geq 0.05$) SEM: Standard Error of Mean

Table 8: Effect of dietary supplementation and *in ovo* feeding of alpha-ketoglutaric acid on yield of giblets (heart, liver, gizzard) of commercial broilers after 6 weeks of age (% live weight)

Group	Heart %	Liver %	Gizzard %
Un-injected + Basal diet	0.52	2.21	2.72
Un-injected + AKG supplementation	0.64	2.41	3.06
Sham control + Basal diet	0.64	2.41	2.89
Sham control + AKG supplementation	0.59	2.19	2.95
In ovo AKG + Basal diet	0.58	2.29	2.64
In ovo AKG + AKG supplementation	0.61	2.10	2.76
Treatment			
Un-injected	0.58	2.31	2.89
Sham control	0.62	2.30	2.92
In ovo AKG	0.59	2.19	2.70
Diet			
Basal diet	0.58	2.30	2.75
AKG supplementation	0.61	2.23	2.93
Pooled SEM	0.02	0.04	0.10
Significance Level			
Treatment	NS	NS	NS
Diet	NS	NS	NS
Treatment × Diet	NS	NS	NS

NS: Not Significant ($p \geq 0.05$) SEM: Standard Error of Mean

Table 9: Effect of dietary supplementation and *in ovo* feeding of alpha-ketoglutaric acid on cut-up-parts of commercial broilers after 6 weeks of age (% ready to cook yield)

Group	Breast (%)	Back (%)	Wings (%)	Neck (%)	Drumstick (%)	Thigh (%)
Un-injected + Basal diet	25.67	24.01	10.36	5.92	17.24	16.81
Un-injected + AKG supplementation	24.35	27.25	9.33	5.85	16.77	16.46
Sham control + Basal diet	23.29	24.43	10.69	6.27	18.27	17.04
Sham control + AKG supplementation	23.39	24.21	10.67	6.33	17.88	17.52
In ovo AKG + Basal diet	24.62	23.50	10.89	5.94	18.26	16.78
In ovo AKG + AKG supplementation	26.12	24.06	10.42	5.77	16.93	16.70
Treatment						
Un-injected	25.01	25.63	9.84	5.88	17.01	16.63
Sham control	23.34	24.32	10.68	6.30	18.08	17.28
In ovo AKG	25.37	23.78	10.66	5.85	17.60	16.74
Diet						
Basal diet	24.53	23.98	10.65	6.04	17.92	16.87
AKG supplementation	24.62	25.17	10.14	5.98	17.20	16.89
Pooled SEM	0.53	0.44	0.18	0.11	0.22	0.22
Sig Level						
Treatment	NS	NS	NS	NS	NS	NS
Diet	NS	NS	NS	NS	NS	NS
Treatment ×Diet	NS	NS	NS	NS	NS	NS

NS: Not Significant ($p \geq 0.05$) SEM: Standard Error of Mean

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