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SUPPLEMENTATION OF VITAMIN C, VITAMIN E AND ORGANIC CHROMIUM AND ITS COMBINATION IN FEED ON GROWTH PERFORMANCE OF NARMADANIDHI BIRDS DURING SUMMER SEASON

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ABSTRACT

The present study was conducted to study the effect of supplementation of vitamin C (ascorbic acid), vitamin E, organic chromium and its combination in feed on growth performance of Narmadanidhi birds in summer season. A total of 240, day old coloured dual type Narmadanidhi chicks were distributed into 12 dietary treatment groups with 20 chicks in 2 replicates in each treatment. The chicks were housed in individual pens as per treatment groups and reared on litter system. Starter and finisher ration were prepared and fed up to 6 and 7 to 12 weeks of age, respectively. Dietary treatment supplements in starter and finisher ration were C0 control, C1 (150 mg AA/kg), C2 (250 mg AA/kg), E1 (125 mg vit E/kg), E2 (200 mg vit E/kg) Cr1 (1.25 mg Cr-propionate/kg), Cr2 (2.0 mg Cr-Propionate/kg). Combined supplements were C2E1, C2E2, C2Cr1, C2Cr2, and C2E1Cr2. The data of growth performance parameters such as body weight, feed intake (FI) and feed efficiency (FE) were recorded to analyse by one way ANOVA. During summer season, dietary supplementation of C2, E2, Cr1, Cr2 had given significantly higher body weight, feed intake and feed efficiency. Among these C2 was significantly superior body weight. All combined supplements significantly improved body weight, feed intake and feed efficiency over single supplement. Among these, C2Cr2, C2E1Cr2 were significantly superior. It was concluded that, supplementation of vitamin C (ascorbic acid), vitamin E, organic chromium and its combination in feed on growth performance reported that combined supplement C2Cr2, C2E1Cr2 had superior growth performance in Narmadanidhi birds in summer season.

Key words : Vitamin C, Vitamin E, Chromium, Narmadanidhi, Growth performance, Summer season, Feed.

Introduction

When a bird's temperature rises above the upper critical limit of the thermoneutral zone, it is deemed to be under heat stress. Since they lack sweat glands, birds mostly lose heat through evaporation, or panting. Birds who don't employ this system to cool their bodies risk dying when the temperature rises above a certain point. Heat stress has a serious negative impact on feed intake, growth rate, feed efficiency, and carcass production. It also alters the carcass's color, pH, and drip loss (Zheng *et al*., 2016) and it produces pale, soft meat exudates (Tankson *et al*., 2001). Numerous physiological changes were brought on by heat stress and the extent of these changes varied. According to several studies, high temperatures have a negative impact on birds' immune systems and cause biochemical alterations like tissue damage and lipid peroxidation (Sahin *et al*., 2009).

A number of dietary and management changes are made to lessen the negative effects of heat stress. Because of its reducing qualities and electron-donating capabilities, ascorbic acid, also known as vitamin C, plays a significant metabolic role. Environmental stresses, particularly heat stress, have been shown to reduce endogenous vitamin C production, increasing the need

for exogenous vitamin C (Khan, 2011 and Mc Dowell, 1989). During the summer, broilers treated with ascorbic acid showed improvements in feed intake, body weight, and feed efficiency, according to several authors (Sahin *et al.*, 2002 and Attia *et al.*, 2009). Vitamin E is a powerful lipid-soluble antioxidant and anti-stress vitamin that preserves cellular integrity by scavenging free radicals and preventing unsaturated lipids in bodily tissue and organ cell membranes from oxidizing. Vitamin E supplementation in broiler diets has been shown to significantly boost body weight gain, feed intake, and feed efficiency (Singh *et al.*, 2016 and Raza *et al.*, 2018). The use of chromium in domestic animal nutrition has been the subject of research in recent decades. Under normal environmental conditions, a diet containing less than 3 mg Cr/kg food promotes deficiency symptoms, while no recommendation is suggested (NRC, 1997). Growth rate and feed efficiency have been found to benefit from dietary chromium (Cupo and Donaldson, 1987 and Toghyani *et al.*, 2006).

Numerous studies have shown that when birds are under environmental stress, they perform noticeably better when they get a combination of vitamin C and chromium (Haq *et al.*, 2016) and vitamin C and vitamin E (Doba *et al.*, 1985 and Attia *et al.*, 2017). Given the aforementioned information and the critical role that vitamins C, E, and chromium play in metabolism under environmental stress conditions, the current study was designed to examine the growth traits of Narmadanidhi birds during the summer months using varying dietary concentrations of ascorbic acid, vitamin E, and chromium as individual and combined supplements in feed.

Materials and Methods

The present experiment was conducted at All India Co-ordinated Research Project on Poultry Breeding, Department of Poultry Science, N.D.V.S.U., Jabalpur, (M.P.). A completely randomized design (CRD) was utilized to conduct present experiment. A total of 240, day old coloured dual type Narmadanidhi sexed chicks (75% Jabalpur colour and 25% native Kadaknath inheritance) with equal numbers of male and females were distributed into 12 dietary treatment groups with each consisting 20 chicks in 2 replicates. The chicks were housed in individual pens as per treatment groups and reared on litter system. Starter ration was prepared containing 21% CP with 2800 Kcal ME/kg and fed up to 6 weeks. Finisher ration was prepared containing 19% CP with 2900 Kcal ME/kg and fed 7 to 12 weeks of age. Dietary treatment supplements in starter and finisher ration were C 0 control, C1 (150 mg AA/kg), C2 (250 mg AA/kg), E1 (125 mg vit-E/kg), E2 (200 mg vit-E/kg) Cr1 (1.25 mg Cr-propionate/kg), Cr2 (2.0 mg Cr Propionate/kg). Combined supplements were C2E1, C2E2, C2Cr1, C2Cr2 and C2E1Cr2. During 0 to 12 weeks treatment trials, data of the growth performance (Body weight, Feed intake and Feed efficiency) were recorded on 4th, 8th and 12th week. Analysis (One way ANOVA) was carried out to study the effect of treatments on production performance (Snedecor and Cochran, 1989).

Results

Body weight (g)

Body weights (g) of birds during summer season are presented in Table 1.

Fourth week of age : Fourth week body weight was non-significantly different between C_2Cr_1, C_2Cr_2, $C_2E_1Cr_2$ whereas C_2Cr_2 and $C_2E_1Cr_2$ were found significantly heaver weight than all other treatment groups. Chicks in C_0 control and E_1 treatment were non-significantly different and these were significantly lower in body weight than all other treatment groups. Among single supplements, body weight of C_2, Cr_1 and Cr_2 were non-significantly different and were significantly higher than all Control C_0, C_1, E_1, E_2 single supplement groups.

Eight-week age : Body weight of C_2Cr_2 (853.7 g ± 10.0) and $C_2E_1Cr_2$ (857.0 g ± 10.8) were non-significantly different. These were significantly higher than control and all treatment groups, following higher body weight attained in C_2Cr_1 and C_2E_2. Among single supplement, C_2 attained higher body weight (745.0 g ± 10.5) with non-significantly different from Cr_2 and C_2E_1 and had significantly heavier birds than all other single supplements groups. Higher level of AA improved body weight significantly over its lower level whereas Vit E and Cr propionate supplement at higher level given improved body weight with non-significant difference.

Twelfth week of age : Final 12th week body weight in C_2Cr_2 (1289.7 g ± 9.3) and $C_2E_1Cr_2$ (1291.2 g ± 9.4) were non-significantly different. $C_2E_1Cr_2$ had significantly heavier birds than control and all other treatment groups, followed by higher body weight recorded for C_2E_2 and C_2Cr_1. Among single supplements, C_2 attained higher body weight and it was non-significantly different from Cr_2 and C_2E_1 and significantly superior from control and all other single supplement groups. C_2 higher level of AA found beneficial over its C_1 lower level. All combined supplement improved body weight significantly over its respective single supplement groups except C_2 was non-significant from C_2E_1.

Feed intake and feed efficiency of Narmadanidhi birds

Feed intake (g) and feed efficiency of birds during summer season are presented in Tables 1 and 2, respectively.

Feed efficiency

Fourth week of age : At 4th week age, cumulative feed intake of C_2Cr_2 (856.2 g ± 3.68) was non-significantly different from $C_2E_1Cr_2$ (851.4 g ± 2.67) and significantly higher than control and all other treatment groups. Feed intake of all combined supplement groups was significantly higher than individual treatment groups. Control C_0 was lower in cumulative feed intake (CFI) per bird (651.3 g ± 11.14) than all other treatment groups. Feed intake of E_2 was significantly higher than E_1 lower level whereas FI of C_2, Cr_2 were non-significant from C_1, Cr_1 lower levels.

Eight weeks of age : Cumulative feed intake of C_2Cr_2 (2658.6 g ± 6.63) and $C_2E_1Cr_2$ (2656.6g ± 13.13) were non-significantly different and significantly higher than all other treatment groups. CFI of C_2E_1, C_2E_2 and C_2Cr_1 were non-significantly different and C_2E_2, C_2Cr_2 were significantly higher than control and all single supplement groups. Feed intake of C_0, C_1, E_1, E_2, Cr_1 were non-significantly different and E_1 was significantly lower in FI than all other treatment groups. Among single supplements, C_2 and Cr_2 were significantly higher in feed intake than C_0, C_1, E_1, Cr_1. FI was higher with C_2, E_2, Cr_2 than C_1, E_1, Cr_1 lower levels with significant difference in AA and Chromium propionate supplements.

Twelfth week of age : Average CFI / Bird in C_2Cr_2 (4643.0 ± 7.97) and $C_2E_1Cr_2$ (4648.0 ± 15.17) were non-significantly different and significantly higher than control and all other treatment groups. CFI of bird in C_2E_1, C_2E_2, C_2Cr_1 and C_2 were non-significantly different and significantly higher than C_0, C_1, E_1, E_2, Cr_1 and Cr_2 treatments. C_2, E_2 and Cr_2 higher levels improved FI significantly over its respective C_1, E_1 and Cr_1 lower levels. Among single supplements, C_2 Shown significantly higher feed intake than C_0 and all other single supplement groups. CFI in control C_0 was non-significantly different from C_1, E_1, E_2 and Cr_1 and these were significantly lower in FI than all other treatment groups.

Cumulative feed efficiency

Fourth week of age : Feed efficiency during 4th week was also not influenced by treatment effect and it ranged between 1.92 ± 0.01 in C_2E_2 to 1.96 ± 0.01 in C_2E_1 and C_2Cr_1 treatments.

Eight weeks of age : During this period cumulative FE of control group C_0 (3.36 ± 0.01) was non-significantly different from C_1, E_1 and significantly inferior than all other treatment groups. Feed efficiency of C_2Cr_1 (3.12 ± 0.01), C_2Cr_2 (3.12 ± 0.01) and $C_2E_1Cr_2$ (3.10 ± 0.01) were non-significantly different and significantly superior than (C_0) control and all other treatment groups. Following superior FE was noted in C_2E_2 (3.16 ± 0.01). Higher level of AA and Vit E significantly improved FE over its C_1 and E_1 lower levels, whereas Cr_1 and Cr_2 were similar in FE. Among single supplement, C_2 (3.31 ± 0.01), E_2 (3.31 ± 0.01) FE was non-significantly different from Cr_2, C_2E_1 and significantly better than C_0, C_1, E_1 and Cr_1 single supplement groups.

Table 1 : Effect of vitamin C, E, organic chromium and its combination on body weight (g) and Bi-weekly cumulative feed intake (g) of Narmadanidhi birds in summer season.

Treatments	Body weight (g)			Bi-weekly cumulative feed intake (g)		
	4th Week	8th Week	12th Week	4th Week	8th Week	12th Week
C_0	334.50^{f}±7.47	695.00^{e}±11.30	1160.50^{e}±8.84	651.34^{h}±11.14	2352.95^{de}±9.75	4293.81^{de}±5.05
C_1	357.00^{de}±7.53	710.50^{de}±11.06	1160.75^{e}±9.69	691.45^{fg}±0.81	2371.35^{de}±9.60	4269.83^{e}±12.78
C_2	371.75^{cde}±7.23	756.50^{c}±10.58	1228.50^{c}±10.20	711.98^{ef}±9.70	2467.57^{c}±4.13	4459.62^{b}±18.86
E_1	351.25^{ef}±7.29	700.25^{e}±10.51	1159.25^{e}±9.43	686.23^{g}±9.57	2338.00^{e}±16.70	4265.12^{e}±14.72
E_2	365.50^{de}±6.86	715.75^{de}±8.97	1186.75^{de}±9.14	711.70^{ef}±6.10	2366.75^{de}±9.45	4330.84^{d}±10.04
Cr_1	371.75^{cde}±6.30	714.00^{de}±9.19	1186.25^{de}±9.06	725.29^{e}±4.01	2384.72^{d}±6.22	4330.68^{d}±5.07
Cr_2	375.50^{cd}±6.10	734.00^{cd}±7.71	1210.25^{cd}±8.93	725.63^{e}±1.88	2444.18^{c}±5.98	4405.53^{c}±12.22
C_2E_1	390.00^{c}±7.57	753.00^{c}±10.40	1227.00^{c}±10.48	756.65^{d}±13.60	2492.40^{bc}±6.30	4490.65^{b}±19.45
C_2E_2	415.50^{b}±8.44	806.25^{b}±10.95	1261.75^{b}±9.29	796.77^{c}±1.61	2542.96^{b}±22.96	4542.48^{b}±30.76
C_2Cr_1	431.75^{ab}±7.43	816.50^{b}±10.41	1263.00^{b}±10.61	831.79^{b}±3.41	2545.88^{b}±4.32	4547.40^{b}±1.80
C_2Cr_2	438.75^{a}±7.83	853.75^{a}±10.06	1289.75^{ab}±9.35	856.28^{a}±3.68	2658.64^{a}±6.63	4643.83^{a}±7.97
$C_2E_1Cr_2$	440.25^{a}±7.20	857.00^{a}±10.82	1291.25^{a}±9.45	851.39^{ab}±1.67	2656.63^{a}±13.13	4648.03^{a}±15.17

^{a,b,c} Means bearing different superscripts in a column differ significantly ($P<0.05$)

Table 2 : Effect of vitamin C, E, organic chromium and its combination on cumulative feed efficiency of Narmadanidhi birds in summer season.

Treatments	Bi-weekly cumulative feed efficiency		
	4th Week	8th Week	12th Week
C_0	1.95±0.01	3.36^{a}±0.01	3.70^{a}±0.01
C_1	1.95±0.01	3.34^{ab}±0.01	3.68^{ab}±0.00
C_2	1.94±0.00	3.31^{c}±0.01	3.63^{d}±0.01
E_1	1.96±0.01	3.34^{ab}±0.00	3.68^{ab}±0.00
E_2	1.95±0.01	3.31^{c}±0.01	3.65^{cd}±0.01
Cr_1	1.96±0.01	3.33^{bc}±0.01	3.65^{cd}±0.00
Cr_2	1.94±0.01	3.33^{bc}±0.01	3.64^{cd}±0.01
C_2E_1	1.94±0.01	3.31^{c}±0.00	3.66^{bc}±0.01
C_2E_2	1.92±0.01	3.16^{d}±0.01	3.60^{e}±0.01
C_2Cr_1	1.93±0.01	3.12^{e}±0.01	3.60^{e}±0.01
C_2Cr_2	1.96±0.01	3.12^{e}±0.01	3.60^{e}±0.00
$C_2E_1Cr_2$	1.94±0.01	3.10^{e}±0.01	3.60^{e}±0.00

^{a,b,c} Means bearing different superscripts in a column differ significantly ($P<0.05$).

Twelth week of age : During 12th week age, FE of C_2E_2, C_2Cr_1, C_2Cr_2 and $C_2E_1Cr_2$ were similar (3.60 ± 0.01) and significantly superior than control and all other treatments groups. Feed efficiency of C_0 control (3.70 ± 0.01) was non-significantly different from C_1, E_1 and significantly inferior than all other treatment groups. C_2, E_2 FE was significantly better than C_1, E_1 lower level whereas Cr_1 and Cr_2 were non-significantly different.

Discussion

Effect of Ascorbic acid (Vitamin C) on body weight

Ascorbic acid supplementation at C_2 level 250 mg/kg diet given significantly higher body weight than C_0, C_1 and other single supplement groups except Cr_2 which has non-significantly different body weight from C_2 group. Dhore *et al.* (2014) reported significantly higher body weight of broiler supplemented AA at level of 150, 200, 250 mg/kg in diet and better response observed in 250mg/kg AA. Kutlu and Forbes (1994) reported depressed growth of broiler under heat stress and found that dietary AA supplementation at level of 250 mg/kg overcome much of the depression and restored metabolic parameters to normal level. Cier *et al.* (1992) reported better response in broiler growth with 150 mg AA/kg diet than higher level (300 and 600 mg AA/kg diet) reared at 44^0C hot humid condition, whereas El Kheir *et al.* (2008) found positive effect in 250 mg/kg vitamin C supplementation for broiler reared in heat stress.

The present study results were in agreement to these authors and confirmed positive effect on growth of birds by supplementing 250 mg/kg AA, which was in agreements with the result of Kutlu and Forbes (1994), EL Kheir *et al.* (2008) and Dhore *et al.* (2014). However, this study revealed non-significant effect at lower level of supplementation (150 mg AA/kg diet) which was in contrary to Cier *et al.* (1992) and Dhore *et al.* (2014). Non-significant effect on growth with lower level of AA might be due to duration and extent of heat stress and related adverse effect on birds along with micro-environment in experimental shed.

Effect of Vitamin E on body weight

Body weight in E_2 (200 mg /kg diet Vit E supplementation) was significantly higher than control and E_1 lower concentration, in both sexes during initial and later age of growth evaluated under summer season. E_1 lower level was non-significantly different from control group in analysed results.

Singh *et al.* (2016), Attia *et al.* (2017) and Raza *et al.* (2018) reported significantly improved growth of broilers supplemented 100mg and 150 mg/kg vitamin E as tocopherol. There are also many reports in literature which concluded that higher level of dietary vit E inclusion (200, 250, 300 mg/kg diet) had better response on performance traits and immune status of broilers (Sheikh *et al.*, 2017). This study result was in agreement with the finding of above authors for positive effect of vitamin E supplemented at level of 200 mg/kg diet but did not in corroboration with them for improved body weight at 125 mg/kg lower level of inclusion as reported by Singh *et al.* (2016) and Attia *et al.* (2017). This variation in requirement might be severity of heat stress effect on birds, composition of diet including presence of unsaturated fatty acids and managemental condition during experiment period. Since the problem trigger by heat stress in bird are multi-factorial effect including less absorption and more excretion of minerals and vitamins as well as presence of fatty acid in diet and degradation of vit E may be the reason for its better effect at higher level of inclusion in diet.

Effect of Chromium on body weight

Body weight of birds improved significantly with dietary inclusion of organic chromium at levels of Cr_1 (1.2 mg chromium propionate/kg diet) and Cr_2 (2.0 mg chromium propionate/ kg diet) than C_0 control group. The result was in accordance with the finding of Toghyani *et al.* (2011) and Hoeck *et al.* (2020), who reported significantly higher body weight of broilers reared under heat stress condition and supplemented organic chromium in diet. The result was in contrary to Anandhi *et al.* (2006) and Debski *et al.* (2004), who reported non-significant

effect of chromium on body weight.

Effect of combined supplementation of vitamin C and E during summer on body weight

Body weight in C_2E_2 combined supplement groups was significantly higher than control C_0, C_2E_1 and all single supplement groups (C_1,C_2, E_1, E_2, Cr_1, Cr_2) at 4, 8 and 12 week of age. C_2E_1 was non-significant from C_2 at 8, 12 weeks of age. C_2E_1 was significantly higher than E_1 and at all growth period. Shakeri *et al.* (2020) reported that vit C and E in combination reduced negative effect of heat stress on growth of broiler better than their individual effect. Ipek and Sahin, (2007) reported higher live weight of quail with 240mg vit E+250 mg AA/kg diet than their individual effect. Similar positive effect was observed by Sahin and Kucuk (2001a) in combined supplement of vit E + C (200 mg/ Kg vit E + 250 mg/kg vit C) during heat stress whereas; Attia *et al*. (2017) found improved body weight at lower level of vit E (100mg/kg diet) with vit C (250mg/kg diet) and probiotic (2g/kg diet) than their individual effect.

Vit C and E have strong relationship and benefit in immune system by increasing macrophage activity, antibody synthesis and humoral activity in poultry (Shakeri *et al*., 2020). As a result of better action of vit C+E in synergism to improved immune system and antioxidant effect, their combined supplementation might have improved performance of birds.

Effect of combined supplementation of vit C with chromium and vitamin E during summer season on body weight

Combined supplement C_2Cr_1, C_2Cr_2 and $C_2E_1Cr_2$ improved body wt over control and single supplement group and C_2Cr_2, $C_2E_1Cr_2$ were non-significantly different in body weight and significantly higher than control and all other treatment groups during 4, 8 and 12 weeks of age. Sahin *et al*. (2003) reported significantly improved body weight in combined supplement of AA and chromium compared to their separate effect during heat stress condition. Watts (1989) reported better antioxidant effect of chromium and vit C in combined supplementation and found beneficial in improving performance of birds.

Effect of Vit C on feed intake

Under summer season, C_2 AA level (250mg/kg of diet) in diet significantly improved feed intake of birds over control during initial and later age growth period. C_1 lower AA level (150mg/kg diet) was non-significantly different in FI from C_0 control group and significantly lower in FI than C_2 in later age of growth.

Kutlu and Forbes (1993) reported significantly increased feed intake of heat stressed broilers ($36^0C \pm 2^0C$, 6-10 hrs/day) in vitamin C supplemented groups and better effect was observed with supplementation of 250 mg AA/kg of diet than higher levels. Similarly, Sahin *et al.* (2003) and Attia *et al*. (2009) reported significantly improved feed intake of heat stressed broilers given 250mg AA/kg of diet. Several other authors also reported significantly increased feed intake by supplementing vitamin C in diet of birds during heat stress (Farooqi *et al*., 2005). Heat stresse causes increased plasma corticosteroids, protein, glucose, sodium, H/L ratio and decreased potassium and lymphoid organ weight (Kutlu and Forbes, 1993; Kumar *et al*., 2014 and Barrio *et al*., 2020). To minimize heat load, bird decreases its feed intake (Sahin *et al*., 2010) in order to decrease metabolic heat generation and due to disturbed physiological process. Several reported literatures shown that ascorbic acid (vitamin C) supplementation during heat stress decreased plasma corticosteroid concentration and other metabolic disorders, which in turn causes improved feed intake of birds (Mc Dowel, 1989; Kutlu and Forbes, 1993 and Sahin *et al*., 2002a). In contrary to present study, result of Barrio *et al*. (2020) reported non-significant effect of vit C on feed intake of birds during summer stress. The result did not support finding of the authors.

Effect of Vit C on feed efficiency

Feed efficiency of birds was significantly improved by supplementation of C_2 level of AA in diet during summer in compared to control and C_1 lower level. C_1 lower level and control group were non-significantly different. During initial 2 and 4 weeks of age effect of vit C on feed efficiency was not observed. In consistent to the present result significantly improved FE was reported in 250 mg AA/ kg in diet of broilers subjected to heat stress condition *i.e.* 36^0C for 4 hrs/day (Kutlu and Forbes, 1993) and at 32^0C (Sahin *et al*., 2003). Farooqi *et al*. (2005) reported better response in higher level of AA in diet (400 mg/kg diet) whereas beneficial effect on FCR at lower level (150 gm /kg diet) was reported by Mckee and Harrison (1995) and Al-Khauzai *et al*. (2010). Barrio *et al*. (2020) reported significantly better FCR of broiler given 200 mg AA/ 1000 litres of drinking water during heat stress condition (35^0 C for 8-13 hrs/day).

In the present study significantly improved feed efficiency with supplementation of 250 mg AA/kg diet was in line with the finding of Kutlu and Forbes (1993), Sahin *et al*. (2003) and Attia *et al*. (2011). However non-significant effect in lower level of supplementation (150 mg AA/kg diet) did not in agreement with Mckee and Harrison (1995) and Al-Khauzai *et al*. (2010), who

reported significantly improved FCR of broiler by supplementing 150 mg AA/ kg diet. Heat stress induces changes in metabolism, decreases FI and digestibility and absorption of nutrients (Attia *et al*., 2009). Ascorbic acid reduces adverse effect of heat stress (Kutlu and Forbes, 1993 and Sahin *et al*., 2002b) and has protective role in pancreatic tissue to oxidative stress and thus helps it to function properly. This might have improved FE of birds in vit C supplementation during heat stress.

Effect of Vitamin E on feed intake

Dietary supplementation of E_2 vitamin (200 mg vit E/ kg diet) was non-significantly different in feed intake from control group during 8 and 12 weeks of age in summer period whereas E_1 lower level (125 mg/kg diet) was non-significantly different from control group during initial and later age. Attia *et al*. (2017) reported non-significant effect of 100mg/kg vit E on FI of broiler, reared under heat stress. EL-gogary *et al*. (2015) reported non-significant effect of vit E at higher level of inclusion (0-400 mg/kg diet). Similarly non-significant effect of vit E supplementation on FI of birds was also reported by Nobakht (2012), Lopes *et al*. (2015) and Pompeu *et al*. (2015). The result was in agreement to these authors. In contrary to present study Alm-EL-Dien *et al*. (2013), Habibian *et al*. (2014) and Singh *et al*. (2016) observed higher FI of broilers by supplementing vit E at levels of 100-150mg /kg of diet during summer stress. The result did not in agreement with these authors and found its effect at initial four-week period in dual purpose colour birds. Habibian *et al*. (2014) reported increased feed intake during initial 3-week growth of broiler supplemented 125mg /kg vit E during heat stress (37^0C; 8hrs/day) was in corroboration for initial growth in this study period.

Effect of Vitamin E on feed efficiency

Feed efficiency was significantly improved in E_2 vitamin supplementation over control group (8 to 12 weeks) and E_1 lower level (8, 12 weeks). E_1 was non-significantly different from control group of birds during initial and later age of growth. Singh *et al*. (2016) and Raza *et al*. (2018) reported significantly improved FE of broilers, supplemented 100 to 150 mg vit E/kg diet during summer stress. Alm-EL-Dein *et al*. (2013) reported that vit E at level of 150 mg/ kg diet and higher level was more beneficial in improving FE during heat stress than 75 and 100 mg /kg lower levels of inclusion. The result was in agreement to these authors and concluded that 200mg/kg wit E in diet significantly improved FE during initial and later age growth period. Although 125 mg /kg lower level could not find beneficial in FE over control group. Alm-EL-Dein *et al*. (2013) reported better effect in 150 mg vit E /kg in diet than 75 mg/kg lower level. Beneficial effect at higher level was also reported by Sheikh *et al*. (2017) during heat stress. Diet composition, severity and duration of heat stress, and birds utilized for experiment (slow/fast growing) might be the reason for variation in dose concentration. However the result did not supported finding of Lopes *et al*. (2015), Nobakht (2012) Pompeu *et al*. (2015) who reported non-significant effect of vit E on FE during heat stress.

Effect of chromium propionate on feed intake

During summer season, feed intake of birds in chromium propionate supplementation at Cr_1 and Cr_2 level was non-significantly different during 4 week of age and significantly higher than C_0 control group. During 8 and 12 week age FI of Cr_2 birds was significantly higher than C_0 control and Cr_1 lower level. In accordance to present result significantly higher FI and improved FE of broiler during summer stress period with dietary supplementation of 2.5 mg Cr-picolinate was reported by Chougule *et al*. (2018). Toghyani *et al*. (2011) found higher FI in heat stressed broiler (33^0C±2^0C) supplemented 1500µg Cr (Cr-chloride, Cr-nicotinate) over control and 500, 1000 mg lower levels. Consistent finding also reported by Sahin *et al*. (2002) for broiler reared at 32.5^0C and supplemented 400,800 and 1200 µg/kg Cr-picolinate in diet. There is several reported literature observed higher FI of birds in dietary Cr supplementation during heat stress (Sahin, *et al*., 2005; Toghyani *et al*., 2006 and Samanta *et al*., 2008). The present result was in agreement to above authors. In contrary some other authors reported non-significant effect of chromium on FI of broiler reared in heat stress condition (Debski *et al*., 2004 and Anandhi *et al*., 2006). The present result did not support finding of these authors.

Effect of cromium propionate on feed efficiency

Feed efficiency of birds was significantly improved during 8 and 12 week of age by supplementation of chromium propionate during heat stress. Cr-propionate at level of Cr_1 (1.2mg/kg diet) and Cr_2 (2.0 mg/kg diet) were non-significantly different at 8 and 12 week of age and significantly better than C_0 control group of birds indicated that Cr_1 lower level found adequate for better feed efficiency at 8 and 12 week of age. Significantly improved feed efficiency in chromium supplementation during heat stress was reported by Kroliczewska *et al*. (2004), Sahin *et al*. (2005), Samanta *et al*. (2008), Ali *et al*. (2018) and Hoeck *et al*. (2020). The study result revealed non-significant effect of Cr-propionate on FE of birds during initial 4-week period and there after lower level (1.2mg/kg diet) was found adequate for significantly

better FE during 12 week of age. In agreement to above finding; Kroliczewska *et al*. (2004) did not observe effect of Cr-picolinate supplementation on FCR of birds up to 3-week period and found significantly improved FCR in 22-42 and 1-42 days period with better effect in 500 µg/kg than 300 µg/kg Cr-picolinate in diet. Similar to our study, Jackson *et al*. (2008) indicated that dietary addition of chromium propionate at level of 200, 400 and 800 µg/kg doses improved feed efficiency and decreased mortality of broiler during heat stress.

The improved feed efficiency in organic chromium supplementation is attributed to its role in beneficial effect in metabolism of nutrients in birds reared under heat stress condition. Chromium deficiency causes disorder in carbohydrate and protein metabolism and reduces insulin sensitivity in peripheral tissue, consequently decreased growth rate and feed efficiency (Lindemann, 1996 and Khan *et al*., 2014). Dietary organic chromium supplementation alleviate heat stress, works as antioxidant and Immuno-modulant thus improve growth rate and FE (Khan *et al*., 2014).

Combined effect of vitamin C, chromium and vitamin C, E, chromium on feed intake and feed efficiency of birds

Combined supplement group C_2Cr_2 and $C_2E_1Cr_2$ were non-significantly different in FI and significantly higher than all other treatment groups. Feed efficiency was not affected in initial 4th week. During 8 and 12 week of age, C_2Cr_1, C_2Cr_2 and $C_2E_1Cr_2$ were non-significantly different in FE and significantly improved FE than control and all other treatment groups. Ali *et al*. (2018) reported that chromium in combination with other antioxidants, significantly improved weight gain and FI of birds. Sahin and Sahin (2002) observed best effect in FI and FE of broiler in 250 mg vitamin C+ 400 µg cromium/kg in diet. Chougule *et al*. (2018) reported significantly improved FE of broiler in combined supplementation of chromium (2.5 mg/kg diet), $MgSO_4$ (2.5 g/kg diet) and vitamin C (250 mg/kg diet) over individual vitamin C and $MgSO_4$ group and found numerically better than chromium group. Abedayo *et al*. (2020) in their experiment reported reduced performance of broiler in higher level of chromium picolinate (1.2mg/kg diet) and its combined supplementation with AA was found to eliminate adverse effect.

Due to better antioxidant potential of vit C+Cr in combined supplementation, they protect tissues to function properly, increase secretion of digestive enzymes, improve digestibility and metabolism of nutrient resulting into better FE and growth of broiler during heat stress (Preuss *et al*., 1997). In the present study significantly better FI and FE in combined supplementation substantiated finding of above authors.

Combined effect of vitamin C and E supplementation on feed intake and feed efficiency of birds

Combined supplementation of AA and vitamin E at C_2E_1 and C_2E_2 levels significantly improved FI over control and their individual supplemental groups during initial and later ages of birds, reared under summer stress. Feed efficiency was significantly improved in C_2E_2 combination over its individual supplement and control groups.

Attia *et al*. (2017) reported significantly improved body weight and FE of broilers in combined supplementation of vitamin C + E and vitamin C + E + Probiotic, reared under heat stress condition (36^{o}C for 7hrs/day). Consistently Sahin and Kucuk (2001b) reported improved body weight and FCR of quail birds, supplemented vitamin C + E (200mg vit C+ 250 mg vit E/kg diet) during heat stress. Samantha *et al*. (2018) reported increased body weight, FI with improved FE of quail reared under summer stress. The present result revealed significantly improved FE in C_2E_2 combined supplement over C_2 and E_2 individual supplement groups. The finding was in line with the Sahin and Kucuk (2001b), Attia *et al*. (2017) and Samantha *et al*. (2018).

Doba *et al*. (1985) reported increased antioxidant activity of vit E in presence of vit C through reducing tocopherol radicals back to their active form of vit E. Ascorbic acid helps to maintain vit E level through reducing degradative metabolism of vit E and thus increase its effectiveness. Sahin *et al*. (2009) and Shakeri *et al*. (2020) reported that vit C+ E work together in protecting lipid and protein from oxidative stress, protect liver and pancreas from oxidative damage and improve their function during heat tress thus improve digestion and utilization of nutrient result in better FI, FE and growth of birds. Vitamin C alleviates adverse effect of heat stress.

Conclusion

In conclusion, during summer season body weights were significantly higher in C2, E2, Cr1, Cr2. All combined supplements significantly improved body weight over single supplements except C2E1. Among all treatment C2Cr2, C2E1Cr2 were significantly better. Feed intake of C2, Cr1, Cr2 was significantly higher. All combined supplement improved in feed intake and among these C2Cr2, C2E1Cr2 were significantly higher. Cumulative feed efficicency of C2E1, Cr1, Cr2 and all combined supplement groups was significantly improved and C2E2,

C2Cr1, C2Cr2, C2E1Cr2 were superior than all treatments. Overall considering body weight, feed intake and feed efficiency combined supplementation of C2Cr2, C2E1Cr2 had superior performance on Narmadanidhi birds in summer season.

Conflict of interests

The authors declare that the research was conducted in the absence of any potential conflict of interest

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