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Impact of vitamin C, vitamin E, and organic chromium-individually and in combination on blood biochemical parameters of Narmadanidhi birds during winter season

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Abstract
The present study was conducted to assess the effect of vitamin C (ascorbic acid), vitamin E, organic chromium and its combination on blood biochemical parameters of Narmadanidhi birds in winter 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 blood biochemical parameters estimation were recorded to analyse by One way ANOVA. During winter season, Serum glucose, cholesterol was significantly decreased in C_2, Cr_1, Cr_2, C_2Cr_1, C_2Cr_2 and $C_2E_1Cr_2$ with better effect in Cr_2, C_2Cr_2, $C_2E_1Cr_2$. Triglycerides were significantly lower in C_2, Cr_1, Cr_2 and all combined supplement and better effect observed in C_2Cr_2, $C_2E_1Cr_2$. Effect of treatments on Serum protein, albumen, serum Na, K was not observed. Hb was improved in C_2, Cr_1, Cr_2, C_2Cr_1. C_2Cr_2 and $C_2E_1Cr_2$. Percent PCV was not affected. It was concluded that, combined supplement C2Cr2, C2E1Cr2 had superior performance in winter season.

Keywords: Vitamin C, Vitamin E, Chromium, Narmadanidhi, Blood-biochemical, Winter season

1. Introduction
The poultry sector is significantly contributing to the growth of India's agricultural economy and has emerged as one of the most dynamic and rapidly growing segments over the past few decades. However, the industry is currently facing multiple challenges, including rising feed costs, emerging infectious diseases, and various health-related issues, all of which demand urgent attention. In addition to these, environmental stresses particularly those related to extreme temperatures in summer and winter also adversely impact poultry health and industry productivity. While numerous studies have highlighted the beneficial effects of dietary supplementation with vitamin C, vitamin E, and chromium in alleviating heat stress in poultry (Sahin *et al*., 2002) [20] there is limited literature available on the impact and mitigation of winter stress. Cold climatic conditions have been reported to induce physiological stress in poultry, significantly affecting their productivity, health, and disease resistance (Phuong *et al*., 2016) [17]. Dhanalakshmi *et al*. (2007) [6] noted that ambient temperatures below 18 °C are sufficient to trigger cold stress in birds. Moreover, cold stress is known to alter immune responses and haematological parameters, as highlighted by Hangalapura *et al*. (2004) [9] and Olfati *et al*. (2018) [16].
Supplementation with certain nutrients, such as vitamin C and chromium, has been shown to counteract the negative effects of cold stress in poultry (Sahin and Sahin, 2001; Blahova *et al*., 2007) [21, 3]. Vitamin E also plays a crucial role as a structural component of biological membranes, enhancing their stability (McDowell, 1989; Yang *et al*., 2013) [13, 24].

Additionally, it supports immune function by increasing lymphocyte levels and reducing heterophils and the heterophil-to-lymphocyte (H/L) ratio (Habibian *et al*., 2014) [8]. Under stressful environmental conditions, poultry often experience reductions in plasma and tissue levels of tocopherol, vitamin C, and vitamin A, while oxidative stress and lipid peroxidation tend to rise due to increased free radical formation (McDowell, 1989; Naziroglu *et al*., 2011) [13, 15]. These stressors also increase the demand for vitamins and minerals, as absorption efficiency and stability decline (Miltenberg, 1999) [14]. Furthermore, combining supplements such as vitamin C with chromium (Haq *et al*., 2016) [10], or vitamin C with vitamin E (Doba *et al*., 1985; Attia *et al*., 2017) [7, 2] has shown enhanced performance outcomes in birds facing environmental stress.

Considering the important role of vitamins C and E, along with chromium, in alleviating the negative impacts of environmental stress on poultry, this study was undertaken to explore their effects during winter seasons. The research specifically focuses on evaluating the influence of individual and combined dietary supplementation of ascorbic acid, vitamin E, and chromium on blood biochemical parameters in Narmadanidhi birds

2. 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 blood biochemical parameters (Serum Glucose, Cholesterol, Triglycerides, Serum Protein, Albumen, Sodium, Potassium, Haemoglobin, Packed Cell Volume) were estimated and recorded on 12th week. Analysis (One way ANOVA) was carried out to study the effect of treatments on production performance (Snedecor and Cochran, 1989) [23].

3. Results

Estimated data of serum biochemical parameters and its Mean sum square during winter season are presented in Table 1 to 4.

3.1 Serum glucose (mg/dl)

During winter season serum glucose level (mg/dl) in C_0 control was non-significantly different from C_1, C_2, E_1, E_2, C_2E_1 and C_2E_2. Among single supplement groups Cr_1 and Cr_2 were non-significantly different and significantly lower in glucose than C_0, C_1, C_2, E_1 single supplement groups. serum glucose level in Cr_2 (238±4.27), C_2Cr_2 (236±3.75), $C_2E_1Cr_2$ (236.0±4.56) were similar with non-significant difference from Cr_1 and significantly lower than all other treatment groups.

3.2 Serum Cholesterol (mg/dl)

Control group C_0 and C_1, C_2, E_1, E_2, C_2E_1 and C_2E_2 were non-significantly different and these were significantly higher in cholesterol level (mg/dl) than all other treatment groups. In these treatments cholesterol value ranged between 166.7±3.25 (C_2E_2) to 176.7±2.69 (C_0). Lowest cholesterol value were obtained in Cr_2 (128.7±4.27), C_2Cr_1 (134.7±2.06), C_2Cr_2 (125.7±2.17) and $C_2E_1Cr_2$ (125.25 ±1.70). Among single supplements, Cr_2 (128.5±4.27) was significantly lower in cholesterol content and its combination as C_2Cr_2 and $C_2E_1Cr_2$ were significantly lower in cholesterol than control and all other treatment groups.

3.3 Triglycerides (mg/dl)

Among single supplements, Cr_1 and Cr_2 and its combination with C_2 i.e. C_2Cr_1, C_2Cr_2, $C_2E_1Cr_2$ were significantly lower in TG compared to control group. Triglyceride (mg/dl) value in C_0 (36.10±1.78) was non-significantly different from C_1 (36.75±1.78), E_1 (34.50±2.47), E_2 (34.25±1.49) and significantly higher than all other treatments. Lowest T.G. was recorded in $C_2E_1Cr_2$ (24.0±1.47) with non-significant difference from C_2Cr_1 and C_2Cr_2 and significantly lower than all other treatment groups.

3.4 Serum protein and albumen (g/dl)

Serum protein was not affected by AA, Vit E and Cr propionate supplementation during winter season and it ranged between 4.58 mg/dl to 4.67 mg/dl. Like Serum protein effect of treatment on serum albumen was not observed and it ranged between 1.24 g/dl to 1.28 g/dl.

3.5 Serum sodium and potassium (mmol/L)

Effect of treatments was not observed on serum sodium and potassium, it was ranged between 135.7±0.48 to 139.5±0.96 and 4.27±0.01 to 4.30±0.01 respectively.

3.6 Blood Hb and PCV (g/100ml)

Blood Hb in Cr_2 (9.74±0.01), C_2Cr_2 (9.77±0.01) and $C_2E_1Cr_2$ (9.77±0.01) were non-significantly different and these were significantly higher in Hb contents than control and all other treatment group. Following significantly higher Hb was observed in Cr_1 and C_2Cr_1 with non-significant difference between them. Hb in C_0, C_1, E_1, E_2, C_2E_1, C_2E_2 were non-significantly different and significantly lower than all other treatment groups. PCV was not affected due to treatments and it was ranged between 28.63 to 28.80%.

4. Discussion

4.1 Effect of vitamin C on blood biochemical parameters

During winter season effect of vitamin C at C_1 and C_2 level of supplementation on serum glucose, cholesterol, triglyceride, total protein, albumen, Na, K, PCV and MDA value were observed non-significantly different from control group. Hb (g/dl) was significantly higher in C_2 level in compared to control group.

Saracila *et al*. (2021) [22] reported non-significant effect of vit C on serum glucose, cholesterol and triglyceride during cold climate. Khukhodziinai *et al*. (2021) [12] reported non-significant effect of 250 mg Vit C /kg diet on hematological

parameters, except decreased glucose level after 60 days during cold climate. Altan *et al*. (2003) [1] reported non-significant effect of Vit C on MDA value of chilled carcass of broiler reared under normal condition and found significantly decreased value under heat stress rearing. In absence of the stress factor, particularly heat stress, physio-biochemical parameters of birds were not usually deviated from normal range hence supplementation of vitamin C has little significance during winter season which synthesized in birds under normal condition of rearing unless very high decreased in ambient temperature.

4.2 Effect of vitamin E on blood biochemical parameters

Effect of vitamin E on serum glucose, cholesterol and triglycerides was observed non-significantly different from control group. Kant *et al*. (2015) [11] reported significantly higher glucose in vit E group, though little difference was observed between them. Serum protein and albumen also found numerically higher in vitamin E supplementation which might be due to some positive effect of vitamin E supplementation during winter season due to its role as antioxidant and immune compatibility and improve health of gut (Qureshi *et al*., 2020) [18], better functioning of liver (Debsky *et al*., 2004) [5], nutrient digestibility (Brenes *et al*., 2008) [4]. Serum Na and K were non-significantly different between treatment and control group.

4.3 Effect of chromium propionate on blood biochemical parameters

In winter season Cr_1 and Cr_2 chromium supplementation significantly decreased blood glucose, cholesterol and triglycerides compared to control group. Khukhodziinai *et al*. (2021)[12] studied the effect of Cr picolinate (400µg/kg) and vit C (200mg/kg) on broiler performance during cold season. They reported decreased blood glucose level in all treatment groups compared to control after 60 days but observed non-significant difference at 30 day of treatment. On the contrary, Rajalekshmi *et al*. (2014) [19] reported non-significant effect of chromium propionate on blood glucose levels of broiler during winter season. Effect of chromium on protein, albumen, Na, K was not observed. Percent PCV was not affected but Hb (g/100ml) was found significantly increased.

4.4 Effect of combined supplementation Vit C, E and chromium on blood biochemical parameters

During winter season, serum glucose, cholesterol and triglycerides in C_2E_1, C_2E_2 combined supplement groups were non-significantly different from C_2, E_1, E_2 and C_0 control groups. serum glucose in C_2Cr_2 and $C_2E_1Cr_2$ was significantly lower than single supplement groups C_2, Cr_2, E_1 and C_0 control, whereas cholesterol and T.G. were observed significantly lower in compared to C_2 and C_0 control and found non-significantly different from Cr_1 and Cr_2. Total protein, albumen, Na, K, PCV were not affected due to single or combined supplementation during winter season. Hb in C_2E_1, C_2E_2 was similar to C_0 control, C_2 and E_2 single supplement groups. Hb in C_2Cr_1, C_2Cr_2 and $C_2E_1Cr_2$ was non-significantly different from Cr_1, Cr_2 and significantly higher than C_2 and C_0 control.

5. Conclusion

It may be concluded that effect of supplementation of vitamin C (ascorbic acid), vitamin E, organic chromium and its combination on blood biochemical parameters of Narmadanidhi birds in winter season showed that blood glucose, cholesterol, triglycerides were significantly affected in winter season. Serum protein, albumen, Na, K was uninfluenced. Hb was improved but % PCV was not affected. Overall Combined supplementation of C_2Cr_2, $C_2E_1Cr_2$ had superior performance on Narmadanidhi birds in winter season.

Table 1: Effect of vitamin C, E, organic chromium and its combination on glucose, cholesterol, triglycerides, protein and albumen level of Narmadanidhi birds in winter season

Treatment	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Protein (g/dl)	Albumen (g/dl)
C_0	252.50^{ab}±4.79	176.75^{a}±2.69	36.00^{a}±1.78	4.58±0.03	1.25±0.01
C_1	250.00^{ab}±2.39	178.75^{a}±1.49	36.95^{a}±0.75	4.62±0.03	1.25±0.01
C_2	250.00^{ab}±4.08	171.25^{a}±4.27	32.00^{bcd}±2.71	4.65±0.05	1.27±0.01
E_1	256.25^{a}±5.54	174.50^{a}±3.33	34.50^{abc}±2.47	4.63±0.05	1.28±0.02
E_2	248.25^{bc}±2.39	171.50^{a}±4.79	34.25^{abc}±1.49	4.62±0.03	1.24±0.01
Cr_1	242.25^{cd}±5.15	142.00^{cd}±3.14	30.75^{cde}±2.17	4.66±0.04	1.24±0.01
Cr_2	238.75^{d}±4.27	128.75^{e}±4.27	30.50^{cde}±1.68	4.67±0.05	1.26±0.01
C_2E_1	255.00^{a}±4.56	168.75^{a}±4.27	32.25^{bcd}±1.31	4.63±0.03	1.28±0.01
C_2E_2	250.00^{ab}±2.50	166.75^{ab}±3.25	32.20^{bcd}±2.47	4.61±0.03	1.24±0.02
C_2Cr_1	242.50^{cd}±4.08	134.75^{de}±2.06	26.50^{def}±1.19	4.62±0.03	1.24±0.01
C_2Cr_2	236.25^{d}±3.75	125.75^{e}±2.17	25.25^{ef}±1.80	4.61±0.04	1.26±0.02
$C_2E_1Cr_2$	236.00^{d}±4.56	125.25^{e}±1.70	24.00^{f}±1.47	4.58±0.03	1.26±0.01

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

Table 2: Means sum of squares for glucose, cholesterol, triglycerides, protein and albumen level of Narmadanidhi birds in winter season

Source	DF	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Protein (g/dl)	Albumen (g/dl)
Treatment	11	284.47**	1763.79**	102.14**	0.00	0.01
Error	36	68.4	43.47	13.85	0.00	0.00
Total	47					

Table 3: Effect of vitamin C, E, organic chromium and its combination on sodium, potassium, Hb and PCV level of Narmadanidhi birds in winter season

Treatments	Sodium (mmol/L)	Potassium (mmol/L)	Hb (g/100ml)	PCV (%)
C_0	139.50±0.96	4.28±0.01	9.53^{d}±0.01	28.78±0.05
C_1	138.25±0.63	4.28±0.01	9.54^{d}±0.02	28.74±0.06
C_2	138.75±0.75	4.28±0.01	9.57^{c}±0.00	28.66±0.06
E_1	138.25±0.63	4.29±0.02	9.54^{d}±0.01	28.63±0.09
E_2	138.00±1.08	4.29±0.02	9.53^{d}±0.01	28.70±0.07
Cr_1	138.75±0.63	4.28±0.01	9.65^{b}±0.01	28.71±0.04
Cr_2	138.25±0.63	4.28±0.01	9.74^{a}±0.01	28.76±0.04
C_2E_1	138.75±0.75	4.28±0.01	9.52^{d}±0.01	28.80±0.04
C_2E_2	136.25±0.75	4.27±0.01	9.53^{d}±0.01	28.79±0.04
C_2Cr_1	135.75±0.48	4.29±0.01	9.67^{b}±0.01	28.71±0.03
C_2Cr_2	139.00±1.29	4.30±0.01	9.77^{a}±0.01	28.76±0.06
$C_2E_1Cr_2$	138.50±0.96	4.29±0.01	9.77^{a}±0.01	28.76±0.05

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

Table 4: Means sum of squares for sodium, potassium, Hb and PCV level of Narmadanidhi birds in winter season

Source	DF	Sodium (mmol/L)	Potassium (mmol/L)	Hb (g/100ml)	PCV (%)
Treatment	11	4.79	0.00	0.04**	0.01
Error	36	2.73	0.00	0.00	0.01
Total	47				

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