Effect of maternal dietary manipulation and in ovo injection of nutrients on the hatchability indices, post-hatch growth, feed consumption, feed conversion ratio and immunocompetence traits of turkey poultgs

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ABSTRACT

Two hundred turkey breeder hens and 24 viable toms of 30-35 weeks (wk) old of small white variety were distributed into 2 treatment groups having 4 replicates First four replicates were offered diet A [National Research Council. 1994. Nutrient requirements of poultry. 8th ed. Washington, DC: National Academy Press] and other four replicates were maintained on a higher plane of nutrition – diet B for 8 wk. Five hundred and fortyeight fertile eggs on 21st embryonic day were in ovo injected with nutrients (essential amino acids - INA; linolenic acid, linoleic acid, retinol and DL-alpha-tocopherol-INFV; INA + INFV-INA VF, sham control – S and un-injected control – C). INAFV poultgs had significantly (P < .01) higher body weight compared to other treatment groups till 8 wk of age. Total immunoglobulins in response to 1% sheep red blood cells were significantly higher (P < .01) in the INA group compared to the C group. Cell-mediated immune response was significantly higher (P < .01) in the diet B group compared to diet A group. Thus, INAFV treatment may be carried out for better posthatch growth and breeders may be maintained on higher plane of nutrition along with INA treatment to elicit better post-hatch immunity.

1. Introduction

Over the past few years, a lot of changes have taken place in the modus operandi of turkey feeding, breeding and management. Genetic improvements in growth have been associated with health-related problems in modern turkey. Nutrition can modulate quantitative and qualitative aspects of the immune response to pathogens. Research on poultry, especially in chickens, has elucidated the impact of diet on immune competence (Cook 1991; Koutsos & Klasing 2001) and the mechanisms that are responsible. Proper functioning of the immune system depends upon availability of essential nutrients, the precursors for cell growth and activity. In fact, branched chain amino acids such as isoleucine, leucine and valine have the greatest potential to modulate immune responses among the amino acids in chickens (Konashi et al. 2000). Further, it has been reported that methionine supplementation in diet results in significant dose-related increases in total antibody immunoglobulin G (IgG) and responses to phytohaemagglutinin from Phaseolus vulgaris (PHA-P) (Tsiagbe et al. 1987). It has also been observed that maternal dietary lipids alter bone development by influencing organic matrix quality and mineralization in embryos (Liu et al. 2003). In another study, Klasing (1998) pointed out that vitamins like A, D and E supply substrates to the immune system, bind to intracellular receptors and modify the release of secondary messengers, thereby regulating the action of leucocytes.

Few days pre- and post-hatch is a critical period for the development and survival of turkeys. Maintenance of glucose homeostasis is one of the major physiological processes during late embryonic phase and this is achieved primarily by glycogen from liver and gluconeogenesis from protein mobilized from amnion, albumen and ultimately muscle. However, logistical problems in incubation may limit oxygen availability to the embryo and thereby lead to low glycogen status and more mobilization of muscle protein towards gluconeogenesis restricting growth and development of embryo and hatching. Efforts have already been made to increase the supply of glucose to reduce gluconeogenesis and free amino acids for higher protein synthesis by injecting carbohydrates and amino acids directly into the egg. It has been reported that injecting amino acid solution into the yolk sac at 7th day (d) of incubation in broiler breeder eggs had no adverse effect on hatchability and body weight (BW) of chicks increased relative to egg weight prior to incubation (Ohta et al. 1999). At the same time nutrients administered into the yolk sac of developing embryo might also act as early source of feed, sparing the precious maternal antibodies and omega fatty acids from being used as nutrients for different developmental activities. Further, in ovo injection of amino acids increased the immunocompetence traits of broiler chicks by increasing substrates required for synthesis of antibodies (Bhanja & Mandal 2005).

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maternal dietary regimen and in ovo nutrient administration on the post-hatch immunocompetence traits of turkey poults.

2. Materials and methods

2.1. Experimental design

Two hundred turkey breeder hens and 24 viable males (toms) of 30–35 weeks (wk) of age were distributed into 2 treatment groups having 4 replicates of 25 hens and 3 toms in each treatment. First four replicates were offered turkey breeder diet, diet A (NRC1994) and other four replicates were maintained on a higher plane of nutrition, diet B (Tables 1 and 2). The birds were housed in a deep litter system. Fertile eggs were collected by natural mating with 10 (hens):1 (tom) ratio. Five hundred and forty-eight fertile eggs were collected and divided into 4 subgroups and weighed and were stored at 15°C for incubation and further treatment.

2.2. In ovo injection

In ovo injection of nutrients was carried out based on the results of a preliminary experiment on the site, needle length and days of embryonic age (Bhattacharyya et al. 2012). On the 21st embryonic day, the eggs were in ovo injected with nutrients (1 ml of nutrient solution/egg) through a pinhole made at the narrow end of the egg with a 24G hypodermic needle (25 mm long) to reach the yolk sac. The procedure was carried out in a laminar flow system and the pinhole site was sealed with sterile paraffin wax immediately after injection. Eggs were returned to the incubator and transferred to a hatcher on the 25th embryonic day and placed in pedigree hatching boxes. On the 29th d unhatched eggs were broken for visual examination to ascertain the cause and age of death by comparing these with stages of embryo development described by Hamburger and Hamilton and expressed as percentage of total fertile eggs set.

2.3. Preparation of nutrient solution

The amino acid composition of egg reported by Ohta et al. (2001) was taken as standard for the preparation of amino acid solution. The concentration of amino acids in the eggs used in the experiment was calculated on the basis of egg weight (Table 4). The study was conducted using crystalline amino acids. 0.3 mg of retinol, 10 mg of DL-alpha-tocopherol and 50 mg each of linoleic and linolenic acid were injected per egg. The nutrients were dissolved in 5% ethanol (prepared in double distilled water), which also was the sham control. The final pH was checked before injection and maintained around 6 (by adding few drops of dilute NaOH/HCl in the stock solution). Prior to in ovo injection, the solutions were warmed to 30°C.

Table 1. Gross composition of diet A and diet B.

| Feed ingredients       | diet A (%) | diet B (%) |
|------------------------|------------|------------|
| Maizea                 | 63.6       | 62.8       |
| Deoiled rice branb     | 12.6       | 7.15       |
| Soybean mealb          | 7.5        | 14         |
| Fish mealc             | 5          | 5          |
| Sunflower mealc        | 0          | 1.1        |
| Linseed oilc           | 0          | 1.65       |
| Lardd                  | 3          | 0          |
| Dicalcium phosphatec   | 1.5        | 1.5        |
| Limestonec             | 6.25       | 6.25       |
| Trace mineral premixd  | 0.05       | 0.05       |
| Vitamin premixd        | 0.07       | 0.07       |
| Lysinee                | 0.03       | 0          |
| Retinolb               | 0          | 2.75       |
| DL-alpha-tocopherolc   | 0          | 145        |
| Ascorbic acidc         | 0          | 150        |
| Zinc sulphatec         | 0          | 125        |
| Sodium Selenitec       | 0          | 1.3        |
| Choline Chloridec      | 0.1        | 1          |
| Saltc                  | 0.3        | 3          |

*pExpressed as %.

Table 2. Nutrient composition of diet A and diet B of turkey breeders.

| Characteristics          | Unit       | diet A (%) | diet B (%) |
|--------------------------|-----------|------------|------------|
| MEa                      | MJ/kg     | 12.14      | 12.14      |
| CPb                      | %         | 14.04      | 16.13      |
| Linoleic acidc           | g/kg      | 12.4       | 22.6       |
| Linolenic acidc          | g/kg      | 0.8        | 10.2       |
| Retinolb                 | mg/kg     | 2.2        | 4.95       |
| Dl-alpha-tocopherolc     | mg/kg     | 35.17      | 199.86     |
| Ascorbic acidc           | mg/kg     | 0          | 150        |
| Zincc                    | mg/kg     | 59.52      | 118.06     |
| Seleniumc                | mg/kg     | 0.2        | 0.52       |
| Calciumc                 | g/kg      | 30.8       | 31         |
| Available Phosphorousc   | g/kg      | 5.5        | 5.5        |
| Lysinee                  | g/kg      | 6.2        | 7.4        |
| Methioninec              | g/kg      | 2.7        | 2.9        |
| Arginineb                | g/kg      | 8.1        | 9.8        |
| Threoninec               | g/kg      | 5.1        | 5.9        |
| Tryptophanc              | g/kg      | 1.4        | 1.8        |
| Isoleucinec              | g/kg      | 5.5        | 6.6        |
| Leucinec                 | g/kg      | 14.2       | 15.8       |
| Phenylalaninec           | g/kg      | 6.9        | 8.0        |
| Valinc                    | g/kg      | 6.9        | 7.9        |
| Histidinec               | g/kg      | 3.8        | 4.4        |
| Glynec                    | g/kg      | 5.9        | 6.2        |

*pCalculated values.

Table 3. Composition of basal diet.

| Characteristics          | Unit       | (%)  |
|--------------------------|-----------|------|
| Maize                    |           | 42   |
| Soybean meal             |           | 43.75|
| Fish meal                |           | 8    |
| Animal fat               |           | 2.25 |
| Dicalcium phosphate      |           | 2    |
| Limestone powder         |           | 1    |
| Mineral mixture          |           | 0.1  |
| Vitamin mixture          |           | 0.025|
| Choline chloride (60%)   |           | 0.16 |
| Salt                     |           | 0.1  |
| Methionine               |           | 0.1  |
| Chemical composition     |           | (%)  |
| Crude protein            |           | 28   |
| Metabolizable energy (MJ/kg) |   | 11.71|
| Lysined                  |           | 1.25 |
| Methionine               |           | 0.5  |
| Calcium                  |           | 1.65 |

*Each (g) contains: copper – 15 mg, iron – 250 mg, iodine – 6 mg, manganese – 300 mg and zinc – 300 mg.

*Each (g) contains: vitamins A – 82,500 IU, B1 – 50 mg, D3 – 12,000 IU, K – 10 mg, B6 – 8 mg, B12 – 16 mg, B12 – 80 mg, E – 80 mg, niacin – 120 mg, calcium pantothenate – 80 mg.

*Analysed values.

*Calculated values.
All the turkey chicks hatched from the respective groups were reared in electrically heated battery brooders and provided a ration having 28% CP and 11.71 MJ/kg up to 8 wk of age (Table 3).

### 2.4. Hatchability attributes production performance and immune response

Hatch weight, percent hatchability, biweekly BW, feed consumption and feed conversion ratio (FCR) were recorded till 8 wk of age. At 4 wk of age, humoral immune response was studied. SRBC (sheep red blood cells) suspended in Alsever’s solution were washed three times in isotonic phosphate-buffered saline (PBS; pH 7.2) using centrifugation (700 × g) and adjusted to provide a 1% suspension (v/v) which was stored at 4°C prior to use. Six 4 wk poult from each treatment were injected intravenously with 1 ml of the SRBC suspension. Five days later, a blood sample (2 ml) was obtained from the jugular vein of each poult. Each blood sample was allowed to clot for serum collection and sera were stored at −20°C until analysis. The antibody response to SRBC was determined using a standard hemagglutination assay (Siegel & Gross 1980; van der Zijpp1983). Further, 2-mercaptoethanol-resistant antibodies, IgG and mercaptoethanol-sensitive antibodies, immunoglobulin M (IgM) against SRBC were determined using hemagglutinations assay (Martin et al. 1989). Cell-mediated immune response was assessed in 120 poult using the in vivo cutaneous basophilic hypersensitivity response to the lectin PHA-P. Toe web thickness, between the third and fourth digits of both the left and right feet was measured using a micrometer. Thereafter, 100 µg PHA-P, dissolved in 0.1 ml PBS, was injected into the same interdigital space of the right foot. The toe web of the left foot was used as the control and injected only with PBS. The inflammatory response was determined 24 h later by measuring the thickness of the respective toe webs and subtracting the earlier measurements using the formula (Corrier & DeLoach 1990). Foot web index = \((R_2−R_1)−(L_2−L_1)\) where \(R_2 = \) thickness 24 h after PHA-P injection, \(R_1 = \) thickness before injection of PHAP-P injection, \(L_2 = \) thickness 24 h after PBS injection and \(L_1 = \) thickness before PBS injection. Phagocytic activity was evaluated as per the method of Cheng and Lamont (1988). India ink was centrifuged (700 × g) for 45 min and supernatant fraction was collected. This supernatant fraction (carbon) was injected in brachial vein @ 1 ml/kg BW of experimental birds. Three blood samples of 200 µl were collected in 4 ml of 1% sodium citrate solution from the opposite wing of each bird before injection, after 3 and 15 min of carbon injection respectively. These samples were centrifuged and the relative amount of carbon remained in the supernatant was measured by spectrophotometer in red light (675 nm). The phagocytic indices were calculated as the negative of slope of line determined between time and optical density readings.

### 2.5. Statistical analysis

Data obtained from the above experiment were subjected to 2 × 4 factorial analysis of variance in a completely randomized design (Snedecor & Cochran 1994). Significant differences among treatment means were calculated as per Duncan’s multiple range test (Duncan 1955).

### 3. Results and discussion

#### 3.1. Hatchability attributes

Overall, irrespective of the plane of breeder nutrition, percent hatchability was highest in the INFV-injected group among the in ovo nutrient-injected groups (Table 5). Further, percent hatchability was highest in the diet A group subjected to INFV followed by the diet B group and subjected to INA (Table 6). The high hatchability in the diet A group and subjected to INFV injection might be due to the positive effect of linolenic acid. Vilchez et al. (1992) reported adding linolenic acid to basal diet increased hatchability and decreased late embryonic mortality. This corroborated with our studies as there was no death after pipping. The higher hatchability in the high immune group and subjected to in ovo amino acid injection might be due to the availability of free amino acid through in ovo injection that might have stimulated embryonic gluconeogenesis which in turn helped hatching activities. In normal circumstances, the embryos use their energy reserves to meet the high demand for glucose to fuel hatching activities (Freeman 1965; John et al. 1987; Christensen et al. 2001). Glucose is primarily generated from protein by gluconeogenesis or glycolysis of glycogen reserves because oxygen is limited during the last quarter of incubation (Bjones et al. 1987; John et al. 1987). However, late term embryo mostly

### Table 4. Amino acid composition of egg and injected solution.

| Amino acid | 61 g | 80 g | Relative to lysine | 2% concentration | Concentration of nutrients for 100 eggs (mg) |
|------------|------|------|-------------------|------------------|------------------------------------------|
| Lys        | 584.39| 766.41| 100               | 15.3282          | 1532.82                                   |
| Met        | 294.95| 386.82| 50.47             | 7.7364           | 773.64                                    |
| Arg        | 301.30| 567.44| 85.78             | 13.1488          | 1314.88                                   |
| Thr        | 391.25| 513.11| 66.94             | 10.2622          | 1026.22                                   |
| Ileu       | 419.40| 550.03| 71.77             | 11.0006          | 1100.06                                   |
| Leu        | 700.41| 918.57| 88.34             | 13.5408          | 1354.08                                   |
| Val        | 516.24| 677.04| 88.34             | 13.5408          | 1354.08                                   |
| Trp        | 169.34| 153.35| 20.01             | 3.067            | 306.7                                     |
| His        | 209.34| 274.54| 35.82             | 5.4908           | 549.08                                    |
| Gly        | 274.14| 359.53| 46.91             | 7.1906           | 719.06                                    |

#### Notes: NS: not significant (P > .05). SEM: standard error of means.

### Table 5. Effect of breeder diet manipulation and in ovo injection of nutrients on hatchability indices of turkey egg.

| Diet | Death before pipping (%) | Death after pipping (%) | Hatchability (%) |
|------|--------------------------|-------------------------|------------------|
| Diet A | 26.44                  | 5.48                    | 68.08            |
| Diet B | 32.15                  | 5.75                    | 62.1             |
| Treatment |                      |                          |                  |
| INA   | 35.54                  | 5.24                    | 59.23            |
| INVF  | 32.27                  | 2.5                     | 65.23            |
| INFV  | 44.6                   | 7.06                    | 46.33            |
| S     | 24.07                  | 8.63                    | 67.30            |
| C     | 10                     | 4.65                    | 85.35            |
| Pooled SEM |            | 0.95                    | 4.60             |

#### Notes: not significant (P > .05). SEM: standard error of means.
depends on gluconeogenesis from amino acids (Dickson & Langslow 1978; John et al. 1988; Hamer & Dickson 1989).

Among all the in ovo treatment groups, irrespective of the breeder plane of nutrition, chick weight to egg weight ratio was significantly higher (P<.05) in the in ovo amino acid along with fatty acid and vitamin-injected group and in ovo amino acid-injected group compared to in ovo fatty acid and vitamin-injected group and apparently higher compared to other groups (Table 7). Uni et al. (2005) reported that in ovo feeding to the late term embryos increased the hatching weight by 5–6% over controls. In other studies, Ohta et al. (2001) and Bhanja and Mandal (2005) reported that in ovo administration of all 20 amino acids increased the chick weight by 3.6% and 2.1%, respectively. They opined that in ovo administration of amino acids might have stimulated the amino acid utilization and concomitant decrease in amino acid degradation by the embryo. Free amino acids have also been found to decrease protein degradation in the hepatocytes of rats (Mortimore et al. 1991; Venerando et al. 1994).

In the present study, amino acid group and amino acid along with fatty acid and vitamin had around 1.5% higher chick weight to egg weight ratio compared to un-injected control (Bhanja & Mandal 2005). In the present study, amino acid group and amino acid along with fatty acid and vitamin-injected group compared to in ovo fatty acid and vitamin-injected group and apparently higher compared to other groups (Table 7). Uni et al. (2005) reported that in ovo feeding to the late term embryos increased the hatching weight by 5–6% over controls. In other studies, Ohta et al. (2001) and Bhanja and Mandal (2005) reported that in ovo administration of all 20 amino acids increased the chick weight by 3.6% and 2.1%, respectively. They opined that in ovo administration of amino acids might have stimulated the amino acid utilization and concomitant decrease in amino acid degradation by the embryo. Free amino acids have also been found to decrease protein degradation in the hepatocytes of rats (Mortimore et al. 1991; Venerando et al. 1994).

The chick weight to egg weight ratio in the amino acid-injected group compared to in ovo fatty acid and vitamin-injected group was significantly higher (P<.05) in the present study. A study by Bhanja et al. (2006) also revealed that there was no difference in chick weight and egg weight, but their ratio was higher in 0.25 IU vitamin E and 25 mg linoleic acid-injected chicks than un-injected control. However, in the present study there was no difference in chick weight and its ratio to egg weight, which might be due to in ovo injection of higher dose of both vitamin E (10 IU) and linoleic acid (50 mg).

Interaction of maternal dietary manipulation and in ovo injection of nutrients on chick weight revealed no significant difference among the treatment groups (Table 8). In addition, there was no significant difference in chick weight to egg weight ratio among the different treatment groups.

### 3.2. Growth

Poults hatched from diet B had significantly higher (P<.05) BW compared to those hatched from diet A group at 4 wk of age and apparently higher BW throughout the experiment. Further, INAFV poults had significantly (P<.01) higher BW compared to other treatment groups from 2 wk onwards (Table 9). In addition, INAFV poults subjected to either of the breeder diet

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**Table 6.** Interaction of breeder diet manipulation and in ovo injection of nutrients on hatchability indices of turkey egg.

| Group | Death before pipping (%) | Death after pipping (%) | Hatchability (%) |
|-------|--------------------------|-------------------------|------------------|
| Diet A | 41.07                    | 7.14                    | 51.79            |
| INA   | 27.87                    | 0                       | 72.13            |
| INAFV | 44.2                     | 7.46                    | 48.33            |
| S     | 19.05                    | 6.35                    | 74.60            |
| C     | 0                        | 6.45                    | 93.55            |
| Diet B | 30                       | 3.33                    | 66.67            |
| INA   | 36.67                    | 5                       | 58.33            |
| INAFV | 45                       | 6.67                    | 48.33            |
| S     | 29.09                    | 7.49                    | 60               |
| C     | 20                       | 2.86                    | 77.14            |
| Pooled SEM | 4.36                  | 0.95                    | 4.60             |

**Table 7.** Effect of breeder diet manipulation and in ovo injection of nutrients on poult weight (g) and their ratio (%).

| Diet | Egg weight | Chick weight | Ratio |
|------|------------|--------------|-------|
| Diet A | 79.44      | 49.23        | 61.95 |
| Diet B | 78.51      | 48.55        | 61.81 |

**Table 8.** Interaction of breeder diet manipulation and in ovo injection of nutrients on poult weight (g) and their ratio (%).

| Group | Egg weight | Chick weight | Ratio |
|-------|------------|--------------|-------|
| Diet A | 78.88      | 50.53        | 64.02 |
| INFV  | 78.95      | 48.33        | 61.21 |
| INAFV | 81.45      | 50.11       (1.11) | 61.51 |
| S     | 79.44      | 48.89        | 61.51 |
| C     | 79.82      | 49.51        | 62.03 |
| Diet B | 78.26      | 48.22        | 61.56 |
| INFV  | 77.5       | 47.32        | 60.97 |
| INAFV | 78.62      | 50.01        | 63.63 |
| S     | 78.96      | 48.68        | 61.68 |
| C     | 79.22      | 48.54        | 61.25 |

**Notes:** NS: not significant (P > .05). SEM: standard error of means.

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had comparatively better BW compared to other groups from the 2nd wk onwards (Table 10).

The difference in BW among the INAFV treatment and C group ranged from 17 to 30 g till 4 wk of age (Table 9). Uni et al. (2005) reported that in ovo feeding resulted in 50–60 g increase in BW at 25 d post-hatch. Bhanja et al. (2004) reported that in ovo injection of 20 amino acids at 7 d of embryonic age had also 33.6–41.2 g higher BW at 3 wk of age than control group. In another study, Bhanja and Mandal (2005) reported that in ovo injection of specific amino acids (Ile + Leu + Val or Gly + Pro) had 63.2 g, 60 g and 33.6 g higher BW at 3d, 14 d and 8th wk of age (Table 12). Further, INAFV poults had significantly lower BW at 49 d of age, 0.25 and 0.50 IU in ovo injection vitamin E groups had 105–142 g higher BW than un-injected control. In the present study, inclusion of amino acids, fatty acids and vitamins might have contributed to the higher BW in turkey poults.

### 3.3. Feed consumption

Chicks hatched from diet B group had significantly higher (P < .01) feed consumption compared to those hatched from diet A group during the 2nd and 6th wk of age (Table 11). Among the in ovo nutrient-injected groups, feed consumption was apparently lower in the INA group than other treatment groups till 6th wk of age. Further, diet A group chicks subjected to INAFV had significantly lower (P < .01) feed consumption during 2nd wk and apparently lower feed consumption thereafter till 8 wk of age (Table 12).

### 3.4. Feed conversion ratio

Poults hatched from diet A group had significantly better FCR during 2nd (P < .01) and 6th wk (P < .05) compared to those hatched from diet B group (Table 13). Further, INAFV poults had significantly better FCR during 4th (P < .05), 6th (P < .01) and 8th wk (P < .01) of age than un-injected control group.
Diet A group pouls subjected to INAFV had better FCR throughout the experiment compared to diet B group (Table 14). Bhanja and Mandal (2005) reported that higher feed intake was recorded in the amino acid-injected groups compared to control; though the FCR was not significantly different, a numerically better FCR was observed in the chicks injected with Gly + Pro and Lys + Met + Cys than the control chicks.

In another study, Bhanja et al. (2006) reported that, though statistically no significant difference was observed in FCR but birds injected with vitamin E had better FCR than un-injected control group. This is in agreement with our result where birds injected with vitamin E had better FCR than un-injected control; though the FCR was not significantly different, a numerically better FCR was observed in the chicks injected with Gly + Pro and Lys + Met + Cys than the control chicks.

### 3.5. Humoral immunity

Maternal dietary manipulation did not result in any significant difference in humoral response to SRBC in turkey pouls (Table 15). Bhanja et al. (2006) reported that higher dose of vitamin E (0.75 IU) had lower anti-SRBC response than lower dose (0.5 IU). Since high dose of vitamin E was injected in the present study, there was no significant difference in humoral response to SRBC among the different in ovo injected groups. Total immunoglobulins and mercaptoethanol-sensitive (IgM) antibody titre (log 2) values in response to SRBC were significantly higher ($P < .01$) in all the in ovo injected groups compared to the control group. However, no significant differences were recorded among the different in ovo injected groups (Table 15). Further, maternal dietary manipulation along with in ovo injection of different nutrients did not yield any significant difference among the different groups (Table 16).

### Table 15. Effect of breeder diet manipulation and in ovo injection of nutrients on humoral immune responses (response to 1% SRBC) at 4 wk of age.

| Diet       | Total immunoglobulin | IgG   | IgM   |
|------------|----------------------|-------|-------|
| Diet A     | 12.03                | 3.40  | 8.63  |
| Diet B     | 11.58                | 2.73  | 8.85  |

Notes: NS: not significant ($P > .05$). SEM: standard error of means.

### 3.6. Cell-mediated immune response

Cell-mediated immune response (in vivo PHA-P response as footpad index) was significantly higher ($P < .01$) in the diet B group pouls compared to diet A group pouls (Table 17). Gore and Qureshi (1997) reported that vitamin E supplementation increased lymphocyte proliferative responsiveness to PHA-P and conconavalin-A. This is in agreement with our study as the high immune group (diet B) which comprised vitamin E (DL-alpha-tocopherol) supplementation had significantly better ($P < .01$) response to PHA-P than the diet A group. Further, diet B group pouls subjected to in ovo amino acid treatment had apparently better response to PHA-P compared to other treatment groups (Table 18).

### Table 16. Interaction of breeder diet manipulation and in ovo injection of nutrients on humoral immune responses (response to SRBC) at 4 wk of age.

| Diet       | Total immunoglobulin | IgG   | IgM   |
|------------|----------------------|-------|-------|
| Diet A     | 12.88                | 3.88  | 9.0   |
| Diet B     | 12.25                | 2.88  | 9.37  |

Notes: NS: not significant ($P > .05$). SEM: standard error of means.

### Table 14. Interaction of breeder diet manipulation and in ovo injection of nutrients on biweekly FCR of turkey pouls till 8 wk of age.

| Group | 2nd wk | 4th wk | 6th wk | 8th wk |
|-------|--------|--------|--------|--------|
| Diet A | 2.01^b | 2.02^a | 1.89   | 2.35   |
| INAFV | 1.40^a | 2.54^bc | 1.94   | 2.45   |
| INAFV | 2.80^d | 2.08^a | 1.91   | 1.83   |
| S     | 2.10^b | 2.19^ab | 2.13   | 2.21   |
| Diet B |        |        |        |        |
| INAFV | 1.53^a | 2.36^ab | 1.82   | 2.11   |
| INAFV | 2.21^bc | 2.16^ab | 2.41   | 2.51   |
| S     | 2.43^d | 2.18^ab | 2.35   | 2.50   |
| Diet A-C | 1.65^a | 2.81^c | 2.54   | 2.42   |
| Diet B-C | 1.60^a | 2.42^ab | 2.86   | 2.61   |
| Pooled SEM | 0.12 | 0.05 | 0.06 | 0.06 |
| Diet × treatment | $P < .01$ | $P < .05$ | NS     | NS     |

Notes: NS: not significant ($P > .05$). SEM: standard error of means.

**Mean values with different superscripts in a column differ significantly ($P < .05$).
Table 17. Effect of breeder diet manipulation and in ovo injection of nutrients on cell-mediated immune response (response to PHA-P) and phagocytic index at 4 wk of age.

| Diet | Foot web index (mm) | Phagocytic index |
|------|---------------------|------------------|
| Diet A | 0.30<sup>a</sup> | 0.07 |
| Diet B | 0.46<sup>b</sup> | 0.07 |
| Treatment | | |
| INA | 0.38 | 0.09 |
| INFV | 0.31 | 0.06 |
| INAFV | 0.31 | 0.06 |
| S | 0.31 | 0.06 |
| C | 0.37 | 0.07 |
| Pooled SEM | 0.03 | 0.01 |
| Significance level | | |
| Diet | <P < .01 NS | |
| Treatment | NS NS | |

Notes: NS: not significant (P < .05). SEM: standard error of means.
<sup>a,b</sup>Mean values with different superscripts in a column differ significantly (P < .01).

Table 18. Interaction of breeder diet manipulation and in ovo injection of nutrients on cell-mediated immune response (response to PHA-P) and phagocytic index at 4 wk of age.

| Group | Foot web index (mm) | Phagocytic index |
|-------|---------------------|------------------|
| Diet A | | |
| INA | 0.21 | 0.09 |
| INFV | 0.3 | 0.07 |
| INAFV | 0.22 | 0.07 |
| S | 0.45 | 0.06 |
| C | 0.28 | 0.07 |
| Diet B | | |
| INA | 0.52 | 0.098 |
| INFV | 0.31 | 0.05 |
| INAFV | 0.41 | 0.05 |
| S | 0.57 | 0.06 |
| C | 0.45 | 0.07 |
| Pooled SEM | 0.03 | 0.01 |
| Significance level | | |
| Diet × treatment | NS NS | |

Notes: NS: not significant (P > .05). SEM: standard error of means.

Though, there was no significant difference among the treatment groups, phagocytic index (in vivo carbon clearance) was apparently higher in high immune group pouls and subjected to in ovo amino acid treatment compared to the C group (Table 18).

4. Conclusion

It may be concluded that in ovo feeding of essential amino acids, linolenic acid, linoleic acid, retinol and DL-alpha-tocopherol treatment may be carried out on the 21st embryonic day in turkey eggs for better chick weight to eggs weight, better post-hatch growth and turkey breeders may be maintained on higher plane of nutrition along with in ovo feeding of essential amino acids to elicit better post-hatch immunocompetence traits.

Disclosure statement

No potential conflict of interest was reported by the authors.

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