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Effect of Maternal Dietary Manipulation and *In Ovo* Injection of Nutrients on the Biochemical Attributes and Carcass Quality Characteristics of Post Hatch Turkey Poults

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ABSTRACT

An experiment was undertaken involving maternal dietary manipulation (NRC, 1994-diet A or higher plane of nutrition-diet B), *in ovo* nutrient injection (*in ovo* essential amino acids- INA; linolenic acid, linoleic acid, retinol and DL-alpha-tocopherol-INFV; *in ovo* essential amino acids, linolenic acid, linoleic acid, retinol and DL-alpha-tocopherol-INAFV, sham control-S and un injected control-C) in a 2 × 4 factorial design. Two hundred turkey breeder hens and twenty-four viable toms of 30 to 35 weeks of age of small white variety were distributed into two treatment groups comprising of four replicates of 25 hens and 3 toms in each treatment. First four replicates were offered diet A and other four replicates were maintained on diet B for eight weeks. Five hundred and forty eight fertile eggs were collected and on 21st day embryonic day (ED), the eggs were *in ovo* injected with nutrients. Irrespective of the plane of breeder nutrition, SGOT levels were significantly higher ($P<0.01$) in day old chicks *in ovo* injected group compared to control. Similarly, poults subjected to INA had significantly higher ($P<0.01$) uric acid levels at day old compared to other *in ovo* injected groups. However, there were no significant differences observed in other serum biochemical attributes. Further, there was no significant difference among different treatment groups in carcass quality characteristics and cut-up-parts at eight weeks of age. Thus, it may be concluded that *in ovo* injection of nutrients may not adversely affect the serum biochemical attributes of the neonates and carcass quality characteristics of turkey poults.

Keywords: Turkey breeder hens, Diet, Biochemical attributes, Carcass quality

The neonatal growth of chicken, Japanese quail and turkey is under the influence of various factors encompassing nutrition, genetics, hormones, stress and management. Thus, feeding essential nutrients to the hen must be followed with their placement in the egg (Wilson, 1997). Maternal programming can occur during egg formation as the egg nutrients affect the embryo when it consumes the yolk and amniotic fluid prior to hatch (Ferket, 2012). Thereafter, a synchronized progression of events occurs to ultimately realize a viable chick. During the later stages of incubation, embryos use their energy reserves to meet the high demand for glucose to fuel hatching activities. Insufficient glycogen and albumen may force the embryo to mobilize more protein toward gluconeogenesis, thus restricting growth of late term embryo. These early nutrition limitations may be alleviated by the administration of critical "external diet constituents" into the amnion of the late term embryo by "*in ovo* feeding". *In ovo* feeding improves the nutritional status of the hatchling by accelerating enteric development for greater digestive and nutrient absorptive capacity. In recent years, studies on *in ovo* feeding of nutrients in broilers and turkeys have been undertaken to elicit growth (Al Murrani, 1982; Bhanja and Mandal, 2005; Uni *et al*., 2005; Bhattacharyya *et al*., 2007; Kadam *et al*., 2008). However, interaction of breeder diet manipulation vis-à-vis *in ovo* feeding on the biochemical

attributes and carcass characteristics of the post hatch has to be studied along with the growth performance and immunocompetence traits. Thus, the present study was undertaken to study the effect of maternal dietary regimen and *in ovo* nutrient administration on the biochemical attributes and carcass quality characteristics of turkey poults.

MATERIALS AND METHODS

Experimental birds and diets

Two hundred turkey breeder hens and 24 viable males (toms) of 30 to 35 weeks of age were distributed into two treatment groups having four replicates of 25 hens and three toms in each treatment. First four replicates were offered turkey breeder diet, diet A (NRC, 1994) and other four replicates were maintained on a higher plane of nutrition, diet B (Table 1 and 2). The birds were housed in deep litter system. Fertile eggs were collected by natural mating with 10 (hens): 1 (tom) ratio. 548 fertile eggs were collected and divided into 4 subgroups and weighed and were stored at 15°C for incubation and further treatment.

Table 1: Physical composition of diet A and diet B

Feed ingredients	Diet A	Diet B
Maize[1]	636.00	628.00
Deoiled rice bran[1]	126.00	71.50
Soybean meal[1]	75.00	140.00
Fish meal[1]	50.00	5.00
Sunflower meal[1]	0	11.00
Linseed oil[1]	0	16.50
Lard[1]	30.00	0
Dicalcium phosphate[1]	15.00	15.00
Limestone[1]	62.50	62.50
Trace mineral premix[1]	0.50	0.50
Vitamin premix[1]	0.70	0.70
Lysine[1]	0.30	0
Retinol[2]	0	2.75
DL-alpha-tocopherol[2]	0	145.00
Ascorbic acid[2]	0	150.00
Zinc sulphate[2]	0	125.00
Sodium Selenite[2]	0	1.30
Choline Chloride1	1.00	1.00
Salt[1]	3.00	3.00

[1]Expressed as g/kg; [2]Expressed as mg/kg

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

	Unit	Diet A	Diet B
ME[1]	kcal/ kg	2903.18	2904.49
CP[2]	%	14.04	16.13
Linoleic acid[1]	g/kg	12.40	22.60
Linolenic acid[1]	g/kg	0.80	10.20
Retinol[1]	mg/kg	2.20	4.95
DL-alpha-tocopherol[1]	mg/kg	35.17	199.86
Ascorbic acid[1]	mg/kg	0	150.00
Zinc[1]	mg/kg	59.52	118.06
Selenium[1]	mg/kg	0.20	0.52
Calcium[2]	g/kg	30.80	31.00
Available Phosphorous[1]	g/kg	5.50	5.50
Lysine[1]	g/kg	6.20	7.40
Methionine[1]	g/kg	2.70	2.90
Arginine[1]	g/kg	8.10	9.80
Threonine[1]	g/kg	5.10	5.90
Tryptophan[1]	g/kg	1.40	1.80
Isoleucine[1]	g/kg	5.50	6.60
Leucine[1]	g/kg	14.20	15.80
Phenylalanine[1]	g/kg	6.90	8.00
Valine[1]	g/kg	6.90	7.90
Histidine[1]	g/kg	3.80	4.40
Glycine[1]	g/kg	5.90	6.20

[1]Calculated values; [2]Analysed values

In ovo feeding

In ovo injection of nutrients were 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 21st day embryonic day (ED), the eggs were *in ovo* injected with nutrients (1 ml of nutrient solution/ egg) with a 25 mm needle at the narrow end of the egg to reach the yolk sac. 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 3). 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

Table 3: Amino acid composition of egg and injected solution

Amino acid	61g	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	501.30	657.44	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	119.85	18.3714	1837.14
Val	516.24	677.04	88.34	13.5408	1354.08
Trp	116.93	153.35	20.01	3.067	306.70
His	209.34	274.54	35.82	5.4908	549.08
Gly	274.14	359.53	46.91	7.1906	719.06

double distilled water), which was the sham control. All the turkey chicks hatched from the respective group were reared in battery brooders and fed ration having 28% CP and 2800 ME/kg up to 8 weeks of age (Table 4).

Table 4: Physical and chemical composition of basal diet

Gross composition	(%)
Maize	42.00
Soybean meal	43.75
Fish meal	8.00
Animal fat	2.25
Dicalcium phosphate	2.00
Limestone powder	1.00
Mineral mixture[1]	0.10
Vitamin mixture[2]	0.025
Choline chloride (60%)	0.16
Salt	0.10
Methionine	0.10
Chemical composition	(%)
Crude protein[3]	28.00
Metabolizable energy (MJ/ kg)[4]	11.71
Lysine[4]	1.25
Methionine[4]	0.50
Calcium[3]	1.65
Phosphorous (Total)[3]	0.90

[1]Each (g) contains: Copper-15 mg, Iron-250 mg, Iodine-6 mg, Manganese-300 mg and Zinc-300 mg; [2]Each (g) contains: Vitamins A - 82,500 IU, B_2 -50 mg, D_3 – 12,000 IU, K – 10 mg. B_1– 8 mg, B6 – 16mg, B12 – 80 mg, E – 80 mg, niacin – 120 mg, calcium pantothenate – 80 mg; [3]Analyzed values; [4] Calculated values.

Biochemical attributes and carcass quality traits

Plasma blood biochemical (SGOT, SGPT, alkaline and acid phosphatase, protein, and uric acid level) were determined at day old by sacrificing six chicks from each dietary treatment and using commercial kits of Span Diagnostics, India, according to the manufacturer's instructions. At 8 weeks of age, 4 birds from each group were sacrificed to study the carcass quality characteristics.

Statistical analysis

Data obtained from the above experiment were subjected to 2X4 factorial analysis of variance in a completely randomized design (Snedecor and Cochran, 1980). Significant differences among treatment means were calculated as per Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Biochemical Attributes

Un-injected control group had significantly higher ($P<0.01$) serum alkaline phosphatase level compared to the INA injected group (Table 5). Among all the *in ovo* treatment groups in NRC plane of maternal nutrition, serum alkaline phosphatase was significantly lower ($P<0.01$) in the *in ovo* fatty acid and vitamin injected group compared to *in ovo* amino acid, fatty acid and vitamin injected group. This might be due to the reason that metabolism of nutrients in the *in ovo* amino acid, fatty acid and vitamin injected

Table 5: Effect of breeder diet manipulation and *in ovo* injection of nutrients on blood biochemical parameters of turkey poults at day old

Diet	Protein (g/dL)	SGPT (U/L)	SGOT (U/L)	Acid phosphatase (KA Units)	Alkaline Phosphatase (KA Units)	Uric acid (mg/100ml)
Diet A	5.36	31.46	15.92	1.38	70.36	1.97^{a}
Diet B	4.85	33.7	17.40	1.40	69.72	3.38^{b}
Treatment						
INA	4.2	46.95^{c}	17.05^{b}	1.37	68.66^{b}	3.54^{c}
INFV	5.28	24.90^{a}	16.61^{b}	1.39	71.07^{bc}	1.88^{a}
INAFV	4.40	23.09^{a}	17.30^{b}	1.38	73.15^{bc}	1.53^{a}
S	5.11	37.41^{b}	19.60^{b}	1.42	62.26^{a}	2.78^{b}
C	6.5	30.55^{ab}	12.75^{a}	1.40	75.05^{c}	3.63^{c}
Pooled SEM	0.28	2.18	0.69	0.02	1.42	0.25
Diet	NS	NS	NS	NS	NS	P<0.01
Treatment	NS	P<0.01	P<0.01	NS	P<0.01	P<0.01

Means bearing different superscripts in a column differ significantly (P<0.05); NS: Not significant (P>0.05). SEM: Standard error of means

group was slower compared to the group in which only fatty acid and vitamin was injected. As a result lipid was deposited in liver i.e. hepatic lipidosis occurred resulting in an increase in activity of serum alkaline phosphatase. Among all the *in ovo* treatment groups in high immune plane of maternal nutrition, serum alkaline phosphatase was significantly lower (P<0.01) in the *in ovo* amino acid injected group compared to *in ovo* fatty acid and vitamin injected group. This may be again due to the reason that metabolism of nutrients in the *in ovo* fatty acid and vitamin injected group was slower compared to the group in which only amino acid was injected.

Irrespective of the breeder nutrition, poults subjected to INA had significantly higher (P<0.01) uric acid levels compared to the other *in ovo* injected groups. Further, serum uric acid levels were significantly higher (P<0.01) in the poults hatched from breeders maintained on a higher plane of nutrition than the diet A (Table 5). This might be due to higher amount of amino acid metabolism which resulted in the increased formation of uric acid.

Irrespective of the plane of breeder nutrition, SGOT levels were significantly higher (P<0.01) in the chicks in *in ovo* injected group compared to un injected control (Table 5). This might be due to excess stress on the liver to metabolize different amino acids. SGOT level was significantly higher (P<0.05) in the poults hatched from breeders fed either diet A or diet B and subjected to INA compared to other treatment groups (Table 6). Further, no significant difference was recorded among the *in ovo* nutrient injected groups in SGPT levels of poults at day old (Table 5). However, SGPT level of poults from breeders on diet B and subjected to no *in ovo* nutrient injection was significantly lower (P<0.05) compared to other *in ovo* nutrient injected groups (Table 6).

Carcass quality

Percent shrinkage was significantly higher (P<0.01) in the diet A group poults compared to diet B (Table 7). Irrespective of the maternal nutrition, there was no significant difference among the different *in ovo* injected groups in any of the carcass traits. Bhanja *et al.* (2004) also reported that the carcass characteristics and cut-up yields did not vary between AA injected and control birds. So it implies that *in ovo* nutrient administration did not influence the carcass characteristics

In the diet A group, dressing percentage was significantly higher (P<0.01) in the INA treatment compared to INFV and the un-injected control. In the diet B group, dressing percentage was significantly higher (P<0.01) in the INFV treatment group than other group. Evisceration percentage was significantly higher (P<0.05) in diet A group having INA treatment compared to the un-injected control and apparently higher than other groups (Table 8).

Table 6: Interaction of breeder diet manipulation and *in ovo* injection of nutrients on blood biochemical parameters of turkey poults at day old

Group	Protein (g/dL)	SGPT (U/L)	SGOT (U/L)	Acid phosphatase (KA Units)	Alkaline Phosphatase (KA Units)	Uric acid (mg/100ml)
Diet A						
INA	4.60	52.88^{c}	15.21^{b}	1.37	71.39^{bcd}	2.09^{ab}
INFV	5.82	22.75^{ab}	15.29^{b}	1.36	65.20^{b}	1.34^{a}
INAFV	3.93	14.49^{a}	16.46^{b}	1.37	74.92^{cd}	1.81^{ab}
S	6.36	34.09^{b}	16.92^{b}	1.41	69.98^{bc}	2.48^{bc}
Diet B						
INA	3.79	41.01^{c}	18.9^{bc}	1.36	65.93^{b}	4.99^{d}
INFV	4.74	27.06^{ab}	17.92^{bc}	1.42	76.95^{cd}	2.42^{bc}
INAFV	4.88	31.68^{b}	18.14^{bc}	1.38	71.39^{bcd}	1.24^{a}
S	4.16	40.72^{c}	22.27^{c}	1.43	54.54^{a}	3.08^{c}
Diet A-C	6.09	33.10^{b}	15.72^{b}	1.39	70.29^{bc}	2.12^{ab}
Diet B-C	6.91	28.0^{b}	9.78^{a}	1.41	79.81^{d}	5.14^{d}
Pooled SEM	0.28	2.18	0.69	0.02	1.42	0.25
Diet X Treatment	NS	P<0.05	P<0.05	NS	P<0.01	P<0.01

Means bearing different superscripts in a column differ significantly (P<0.05); NS: Not significant (P>0.05). SEM: Standard error of means.

Table 7: Effect of breeder diet manipulation and *in ovo* injection of nutrients on carcass characteristics and giblets at 8 weeks of age

Diet	Shrinkage (%)	Dressing (%)	Eviscerated weight (%)	Heart (%)	Liver (%)	Gizzard (%)
Diet A	3.48^{a}	77.50	65.29	0.56	2.35	3.20
Diet B	2.67^{b}	77.57	66.38	0.56	2.43	3.04
Treatment						
INA	2.33	78.56	67.56	0.58	2.24	3.05
INFV	3.19	77.41	65.01	0.54	2.37	3.04
INAFV	3.39	77.09	65.76	0.58	2.35	3.10
S	2.76	77.56	66.47	0.53	2.54	3.18
C	3.64	77.09	64.36	0.57	2.49	3.21
Pooled SEM	0.22	0.30	0.60	0.007	0.03	0.06
Diet	P<0.01	NS	NS	NS	NS	NS
Treatment	NS	NS	NS	NS	NS	NS

Means bearing different superscripts in a column differ significantly (P<0.05); NS: Not significant (P>0.05). SEM: Standard error of means.

Table 8: Interaction of breeder diet manipulation and *in ovo* injection of nutrients on carcass characteristics and giblets at 8 weeks of age

Group	Shrinkage (%)	Dressing (%)	Eviscerated weight (%)	Heart (%)	Liver (%)	Gizzard (%)
Diet A						
INA	2.83^{abc}	78.82^{d}	68.01^{b}	0.56	2.19	3.10
INFV	4.26^{cd}	75.72^{ab}	62.22^{ab}	0.54	2.35	3.05
INAFV	3.07^{abc}	77.73^{bcd}	66.79^{b}	0.56	2.28	3.16
S	2.33^{ab}	79.40^{d}	67.50^{b}	0.53	2.55	3.37
Diet B						
INA	1.83^{a}	78.31^{cd}	67.10^{b}	0.59	2.30	2.99
INFV	2.12^{ab}	79.09^{d}	67.79^{b}	0.54	2.38	3.03
INAFV	3.65^{bc}	76.59^{abc}	64.93^{b}	0.59	2.40	3.05
S	3.19^{ab}	75.72^{ab}	65.44^{ab}	0.53	2.53	3.0
Diet A-C	5.42^{d}	75.31^{a}	60.81^{a}	0.61	2.40	3.35
Diet B-C	2.31^{ab}	78.42^{cd}	67.01^{b}	0.54	2.56	3.11
Pooled SEM	0.22	0.30	0.60	0.007	0.03	0.06
Diet X Treatment	P<0.01	P<0.01	P<0.05	NS	NS	NS

Means bearing different superscripts in a column differ significantly (P<0.05); NS: Not significant (P>0.05).

Table 9: Effect of breeder diet manipulation and *in ovo* injection of nutrients on cut-up-parts at 8 weeks of age

Diet	Breast (%)	Thighs (%)	Drumstick (%)	Back (%)	Neck (%)	Wings (%)
Diet A	28.16	14.67	15.89	17.95	6.22	17.12
Diet B	26.75	14.32	15.49	20.10	6.56	16.77
Treatment						
INA	26.59	14.77	15.75	19.93	6.37	16.58
INFV	28.20	14.58	15.78	17.77	6.44	17.25
INAFV	27.73	14.24	15.57	19.94	5.84	16.68
S	26.44	14.41	15.51	19.54	6.93	17.17
C	28.19	14.48	15.83	17.98	6.47	17.05
Pooled SEM	0.39	0.21	0.16	0.69	0.19	0.19
Diet	NS	NS	NS	NS	NS	NS
Treatment	NS	NS	NS	NS	NS	NS

Means bearing different superscripts in a column differ significantly (P<0.05); NS: Not significant (P>0.05). SEM: Standard error of means.

Table 10: Interaction of breeder diet manipulation and *in ovo* injection of nutrients on cut-up-parts at 8 weeks of age

Group	Breast (%)	Thighs (%)	Drumstick (%)	Back (%)	Neck (%)	Wings (%)
Diet A						
INA	26.64	13.95^{a}	15.32	20.84	6.37	16.89
INFV	28.82	15.66^{b}	16.51	14.73	7.06	17.22
INAFV	28.17	14.17^{ab}	15.66	19.96	4.94	17.10
S	27.43	14.49^{b}	15.44	19.32	6.27	17.06
Diet B						
INA	26.53	15.60^{b}	16.18	19.03	6.38	16.28
INFV	27.58	13.49^{a}	15.04	20.80	5.82	17.27
INAFV	27.38	14.29^{b}	15.50	19.93	6.56	16.35
S	25.45	14.34^{b}	15.58	19.76	7.6	17.28
Diet A-C	30.24	15.22^{b}	16.71	13.91	6.52	17.40
Diet B-C	26.65	13.92^{a}	15.18	21.03	6.43	16.79
Pooled SEM	0.39	0.21	0.16	0.69	0.19	0.19
Diet X Treatment	NS	P<0.05	NS	NS	NS	NS

Means bearing different superscripts in a column differ significantly (P<0.05); NS: Not significant (P>0.05)

Thigh percentage was significantly higher (P<0.05) in the diet B group poults having INA treatment compared to INFV (Table 10). No significant differences were observed in the other cut-up-parts among different treatment groups (Table 9 and Table 10).

CONCLUSION

Thus, it may be inferred from the study that *in ovo* injection of nutrients may not adversely affect the serum biochemical attributes of the neonates and carcass quality characteristics of turkey poults.

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