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Effect of maternal dietary manipulation and *in ovo* injection of nutrients on the body weight gain, feed conversion ratio, development of lymphoid and digestive organs of 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. Turkey breeder hens (200) and viable toms (24) of 30 to 35 weeks of age of small white variety were distributed into 2 treatment groups comprising 4 replicates of 25 hens and 3 toms in each treatment. First 4 replicates were offered diet A and the other 4 replicates were maintained on a higher plane of nutrition (diet B) for 8 weeks. Fertile eggs (548) were collected and on 21st embryonic day (ED), the eggs were *in ovo* injected with nutrients. *In ovo* nutrient injected chicks had significantly better FCR compared to the control group and INA treatment poults had significantly better FCR compared to the other treatment groups throughout the experiment. Small intestine length was significantly lower in the INAFV treatment group compared to the other treatment groups. Caecal length was significantly lower in the INA treatment group compared to the un-injected control group and the INFV group. Hence, it may be concluded that *in ovo* injection of nutrients may have a positive effect in production indices of turkey poults.

Key words: Body weight gain, Carcass quality, Diet, Turkey breeder hens

The neonatal growth of avian is under the influence of various factors encompassing nutrition, genetics, hormones, stress and management. 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). Insufficient glycogen and albumen will force the embryo to mobilize more protein toward gluconeogenesis, thus restricting growth of late term embryo. These early nutrition limitations may be alleviated 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 and improve bone development in hatchlings (Bhanja and Mandal 2005, Uni *et al*. 2005, Bhattacharyya *et al.* 2007, Kadam *et al.* 2008, Al-Shamery and Al-Shuhaib 2015, Yair *et al*. 2015). However, interaction of breeder diet manipulation vis-à-vis *in ovo* feeding has to be assessed. Thus, the present study was undertaken to study the effect of maternal dietary regimen and *in ovo* nutrient administration on the post-hatch growth performance, development of lymphoid and digestive organs of turkey poults.

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MATERIALS AND METHODS

Experimental birds and diets: Turkey breeder hens (200) and 24 viable males (toms) of 30 to 35 weeks of age were distributed into 2 treatment groups having 4 replicates of 25 hens and 3 toms in each treatment. First 4 replicates were offered turkey breeder diet, diet A (NRC 1994) and other 4 replicates were maintained on a higher plane of nutrition, diet B (Tables 1,2). The birds were housed in deep litter system. Fertile eggs were collected by natural mating with 10 (hens): 1 (tom) ratio. Fertile eggs (548) were collected and divided into 4 subgroups and weighed and were stored at 15°C for incubation and further treatment.

In ovo *feeding: In ovo* injection of nutrients was carried out based on the results of a preliminary experiment on the

Table 1. Gross composition of diets A and B

Feed ingredients	Diet A	Diet B
Maize[1]	636	628
Deoiled rice bran[1]	126	71.5
Soybean meal[1]	75	140
Fish meal[1]	50	50
Sunflower meal[1]	0	11
Linseed oil[1]	0	16.5
Lard[1]	30	0
Dicalcium phosphate[1]	15	15
Limestone[1]	62.5	62.5
Trace mineral premix[1]	0.5	0.5
Vitamin premix[1]	0.7	0.7
Lysine[1]	0.3	0
Retinol[2]	0	2.75
DL-alpha-tocopherol[2]	0	145
Ascorbic acid[2]	0	150
Zinc sulphate[2]	0	125
Sodium selenite[2]	0	1.3
Choline chloride[1]	1	1
Salt[1]	3	3

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

Table 2. Nutrient composition of diets A and 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	12.4	22.6
Linolenic acid[1]	g/kg	0.8	10.2
Retinol[1]	mg/kg	2.2	4.95
DL-alpha-tocopherol[1]	mg/kg	35.17	199.86
Ascorbic acid[1]	mg/kg	0	150
Zinc[1]	mg/kg	59.52	118.06
Selenium[1]	mg/kg	0.2	0.52
Calcium[2]	g/kg	30.8	31
Available phosphorous[1]	g/kg	5.5	5.5
Lysine[1]	g/kg	6.2	7.4
Methionine[1]	g/kg	2.7	2.9
Arginine[1]	g/kg	8.1	9.8
Threonine[1]	g/kg	5.1	5.9
Tryptophan[1]	g/kg	1.4	1.8
Isoleucine[1]	g/kg	5.5	6.6
Leucine[1]	g/kg	14.2	15.8
Phenylalanine[1]	g/kg	6.9	8.0
Valine[1]	g/kg	6.9	7.9
Histidine[1]	g/kg	3.8	4.4
Glycine[1]	g/kg	5.9	6.2

[1] Calculated values; [2] analysed values.

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 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; 50 mg of amino acid solution was injected per egg; 0.3 mg of retinol, 10 mg of DL-alpha-tocopherol and 50 mg each of linoleic and linolenic acid were injected per egg (Table 3). All the turkey chicks hatched from the respective group were reared in battery brooders and fed ration having 28% CP and 2,800 ME/kg up to 8 weeks of age (Table 4).

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	306.7
His	209.34	274.54	35.82	5.4908	549.08
Gly	274.14	359.53	46.91	7.1906	719.06

Table 4. Gross composition of basal diet

Gross composition	(%)
Maize	42
Soybean meal	43.75
Fish meal	8
Animal fat	2.25
Dicalcium phosphate	2
Limestone powder	1
Mineral mixture[1]	0.1
Vitamin mixture[2]	0.025
Choline chloride (60%)	0.16
Salt	0.1
Methionine	0.1
Chemical composition	(%)
Crude protein[3]	28
Metabolizable energy (MJ/ kg)[4]	11.71
Lysine[4]	1.25
Methionine[4]	0.5
Calcium[3]	1.65
Phosphorous (Total)[3]	0.9

[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; B2,50 mg; D3, 12,000 IU; K, 10 mg; B1,8 mg; B_6, 16mg; B_{12}, 80 mg; E, 80 mg; niacin, 120 mg;calcium pantothenate, 80 mg. [3] Analyzed values; [4] Calculated values.

Production indices, development of lymphoid organs and GIT: Biweekly body weight (BW) gain and feed conversion ratio (FCR) were determined till 8 weeks (wk) of age. At 8 weeks of age, 4 birds from each group were sacrificed to study the development of lymphoid organs and

gastrointestinal tract (proventriculus, gizzard, small intestine, large intestine, caeca).

Statistical analysis: Data obtained from the above experiment were subjected to 2×4 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

Production indices: INAFV poults had significantly higher (P<0.01) biweekly body weight gain compared to other treatment groups (Table 5). However, diet A group poults subjected to INAFV had apparently higher body weight gain throughout the experiment (Table 6). Bhanja *et al.* (2004) reported that *in ovo* injection of 20 amino acids had only 77.7 g higher body weight than un- injected control chicks at 6 weeks of age. Bhanja *et al.* (2006) reported that at 49 days of age, 0.25 and 0.50 IU vitamin E groups had 105–142 g higher body weight than un-injected control. Al-Shamery and Al-Shuhaib (2015) reported that *in ovo* feeding of lysine, methionine and vitamin E improved initial weight of broiler chickens. In the present study, inclusion of amino acids, fatty acids and vitamins might have contributed to the higher body weight gain in turkey poults. Poults hatched from diet A group had significantly better FCR till 4 weeks (P<0.05), 4 to 8 weeks (P<0.05) and overall duration of 8 weeks (P<0.01) compared to those hatched from high immune group (Table 7). Further, INAFV poults had significantly better (P<0.01) FCR during 4 to 8 and overall duration of 8 weeks of age compared to the un-injected control group. However, INA poults had comparatively better FCR than other treatment groups till 4 weeks of age. Diet A group chicks subjected to INAFV and diet B chicks subjected to INA treatment had better FCR throughout the experiment compared to the other groups (Table 8). However, diet A and diet B group poults subjected to INA had better FCR compared to other treatment groups till 4 weeks of age. Bhanja and Mandal (2005) reported that 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 birds injected with vitamin E had better FCR than un-injected control group. Bakyaraj *et al.* (2012) reported that FCR was better in amino acids and fatty acids+vitamin groups till 3 weeks of age. This is in agreement with our result where INAFV group contained vitamin E and Gly+Lys+Met as a constituent of *in ovo* injection solution, which might have caused better FCR in that group.

Development of lymphoid organs and GIT: Irrespective of the maternal plane of nutrition, thymus weight was significantly lower (P<0.05) in the INFV group than other

Table 5. Effect of breeder diet manipulation and *in ovo* injection of nutrients on body weight gain (g) of turkey poults during biweekly interval

Diet	Second wk	Fourth wk	Sixth wk	Eighth wk
Diet A	73.4	209.9a	283.6	456.8
Diet B	79.5	219.0b	289.8	379.7
Treatment				
INA	77.0b	215.2b	286.1ab	377.9
INFV	76.8b	213.1b	290.1b	539.1
INAFV	91.5c	231.4c	301.6c	384.8
S	66.5a	203.8a	276.3a	374.1
C	75.1b	214.2b	281.7ab	376.4
Pooled SEM	1.9	2.3	2.3	43.9
Diet	NS	P<0.05	NS	NS
Treatment	P<0.01	P<0.01	P<0.01	NS

Means bearing different superscripts in a column differ significantly (P<0.05).

Table 6. Interaction of breeder diet manipulation and *in ovo* injection of nutrients on body weight gain (g) of turkey poults during biweekly interval

Group	Second wk	Fourth wk	Sixth wk	Eighth wk
Diet A				
INA	65.8ab	199.4a	271.6a	372.2
INFV	75.7abc	212.9abc	292.2bc	635.4
INAFV	98.3d	237.1d	307.5c	387.3
S	64.0a	200.0a	272.2a	372.4
Diet B				
INA	82.5c	223.1bcd	293.4bc	380.8
INFV	78.5bc	213.5abc	286.7ab	378.5
INAFV	84.8c	225.7cd	295.7bc	382.3
S	72.6abc	213.2abc	286.5ab	378.4
Diet A-C	74.0abc	210.0ab	278.5ab	375.1
Diet B-C	76.3abc	218.3bc	284.8ab	377.7
Pooled SEM	1.9	2.3	2.3	43.9
Diet × Treatment	P<0.05	P<0.05	P<0.05	NS

Means bearing different superscripts in a column differ significantly (P<0.05).

Table 7. Effect of maternal dietary manipulation and *in ovo* injection of nutrients on FCR of turkey poults during different phases of growth

Diet	0-4 wk	4-8 wk	0-8 wk
Diet A	2.14a	2.20a	2.19a
Diet B	2.24b	2.30b	2.28b
Treatment			
INA	2.02a	2.06a	2.05a
INFV	2.09a	2.35b	2.27b
INAFV	2.64b	1.98a	2.18ab
S	2.19a	2.25b	2.23b
C	2.16a	2.58c	2.45c
Pooled SEM	0.05	0.05	0.03
Diet	P<0.05	P<0.05	P<0.01
Treatment	P<0.01	P<0.01	P<0.01

Means bearing different superscripts in a column differ significantly (P<0.05).

Table 8. Interaction of maternal dietary manipulation and *in ovo* injection of nutrients on FCR of turkey poults during different phases of growth

Group	0-4 wk	4-8 wk	0-8 wk
Diet A			
INA	2.01a	2.18	2.13ab
INFV	2.04a	2.27	2.20abcd
INAFV	2.37b	1.85	2.01a
S	2.15ab	2.18	2.17abc
Diet B			
INA	2.02a	2.00	2.01a
INFV	2.18ab	2.48	2.38cde
INAFV	2.91c	2.12	2.35bcde
S	2.28ab	2.42	2.37cde
Diet A-C	2.26ab	2.46	2.41de
Diet B-C	2.06a	2.69	2.49e
Pooled SEM	0.05	0.05	0.03
Diet × Treatment	P<0.05	NS	P<0.05

Means bearing different superscripts in a column differ significantly (P<0.05).

Table 9. Effect of breeder diet manipulation and *in ovo* injection of nutrients on development of lymphoid organs of turkey poults at eight weeks of age

Diet	Bursa (%)	Thymus (%)	Spleen (%)
Diet A	0.13	0.07	0.18
Diet B	0.13	0.07	0.21
Treatment			
INA	0.13	0.08b	0.22
INFV	0.13	0.03a	0.19
INAFV	0.14	0.08b	0.20
S	0.12	0.07b	0.18
C	0.13	0.08b	0.21
Pooled SEM	0.005	0.008	0.009
Diet	NS	NS	NS
Treatment	NS	P<0.05	NS

Means bearing different superscripts in a column differ significantly (P<0.05).

in ovo injected groups (Table 9). However, there were no other significant differences in weights of lymphoid organs of different treatment groups. Kadam *et al.* (2008) observed that there were no significant differences in weights of lymphoid organs after *in ovo* injection of threonine in broilers. Bhanja *et al.* (2006) reported that there was no difference in bursa weight due to *in ovo* injection of vitamin E and linoleic acid at graded levels. In our study, there was also no difference in bursa weight between immune response type nutrients and their interaction. Length of the large intestine was significantly higher (P<0.01) in the diet A group poults compared to the diet B poults (Table 10). Small intestine (SI) length was significantly lower (P<0.01) in the INAFV poults compared to the other treatment groups. SI weight was apparently higher in the INA group compared to the control group. In the present study, threonine was a constituent of amino acid solution. Kadam *et al.* (2006) also reported though there was no significant difference in digestive organ weights, relative weights of gizzard, liver and small intestine were apparently higher in 40 mg threonine injected than un-injected control. Caecal length was significantly lower (P<0.01) in the INA group compared to the INFV and C groups (Table 11). Diet A group poults subjected to INAFV treatment had comparatively lower small intestine length than other groups. Similarly, the weight of the large intestine was comparatively lower in diet A group poults subjected to INA treatment than other groups. No significant difference was recorded in the other digestive organs.

These results lead us to conclude that *in ovo* injection of

Table 10. Effect of breeder diet manipulation and *in ovo* injection of nutrients on development of digestive organs of turkey poults at eight weeks of age

Diet	Proventricular weight (g/100g)	Small intestine length (cm/100g)	Small intestine weight (g/100g)	Large intestine length (cm/100g)	Large intestine weight (g/100g)	Caecal length (cm/100g)	Caecal weight (g/100g)
Diet A	0.41	8.93	2.52	0.63a	0.23	1.28	0.66
Diet B	0.43	8.58	2.31	0.48b	0.19	1.32	0.74
Treatment							
INA	0.42	8.71ab	2.54	0.50	0.15	1.18a	0.65
INFV	0.43	8.86b	2.21	0.63	0.22	1.34bc	0.71
INAFV	0.43	8.04a	2.42	0.52	0.22	1.25ab	0.64
S	0.40	9.35b	2.51	0.54	0.24	1.41c	0.74
C	0.44	8.85b	2.37	0.58	0.23	1.33bc	0.78
Pooled SEM	0.008	0.13	0.07	0.03	0.02	0.02	0.026
Diet	NS	NS	NS	P<0.01	NS	NS	NS
Treatment	NS	P<0.01	NS	NS	NS	P<0.01	NS

Means bearing different superscripts in a column differ significantly (P<0.05).

Table 11. Interaction of breeder diet manipulation and *in ovo* injection of nutrients on development of digestive organs of turkey poults at eight weeks of age

Group	Proventricular weight (g/100g)	Small intestine length (cm/100g)	Small intestine weight (g/100g)	Large intestine length (cm/100g)	Large intestine weight (g/100g)	Caecal length (cm/100g)	Caecal weight (g/100g)
Diet A							
INA	0.43	9.21bc	2.52	0.47	0.09a	1.17	0.64
INFV	0.45	9.04bc	2.51	0.75	0.24bcd	1.33	0.67
INAFV	0.43	7.72a	2.48	0.54	0.21abcd	1.24	0.58
S	0.36	9.97c	2.64	0.7	0.34d	1.37	0.71
Diet B							
INA	0.42	8.21ab	2.55	0.52	0.21abcd	1.19	0.68
INFV	0.41	8.68ab	1.90	0.50	0.19abc	1.35	0.76
INAFV	0.44	8.30ab	2.38	0.50	0.23bcd	1.25	0.69
S	0.43	8.74ab	2.37	0.38	0.13ab	1.45	0.77
Diet A-C	0.41	8.63ab	2.44	0.71	0.28cd	1.26	0.74
Diet B-C	0.46	9.02bc	2.32	0.49	0.19abc	1.38	0.80
Pooled SEM	0.008	0.13	0.07	0.03	0.02	0.02	0.026
Diet × Treatment	NS	P<0.05	NS	NS	P<0.01	NS	NS

Means bearing different superscripts in a column differ significantly (P<0.05).

nutrients may have a positive effect pertaining to production performance of turkey poults, and the effect may be more pronounced with *in ovo* injection of amino acids, fatty acids and vitamins.

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