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ANIMAL SCIENCE

Effect of in-ovo administration with two levels of amino acids mixture on the performance of Muscovy ducks

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Abstract

Nutrient administration in-ovo could be considered as an alternative method to improve hatchability and duckling weight followed by better economic performance. On the 12th day of incubation, fertile duck eggs (n= 500) were distributed into 5 groups, each of 100. These were: un-injected control; 0.50 ml distilled water; 0.50 ml amino acids (AA) mixture; 0.75 ml distilled water; and 0.75 ml amino acids mixture. In-ovo injection of 0.50 ml but not 0.75 ml of AA mixture resulted in higher hatchability percentage than un-injected control; however this was not statistically confirmed. In-ovo injection of either 0.50 or 0.75 ml of AA mixture resulted in significantly ($P < 0.05$) higher body weight at hatch, marketing weight for males not for females, and higher feed intake than the un-injected control. There was no significant difference ($P < 0.05$) in feed conversion ratio between in-ovo amino acids ducks and un-injected control during the whole experimental periods. Liver weight as a percentage of body weight was higher ($P < 0.05$) in the in-ovo amino acids injected groups than un-injected control. Lymphoid organs of 0.50 AA-injected male group and 0.75 ml AA-injected female group were significantly ($P < 0.05$) heavier than the un-injected control. Antibodies titers did not differ ($P < 0.05$) between in-ovo amino acids injected groups and un-injected control. It is concluded that in-ovo injection of amino acids mixture may improve and accelerate growth and post-hatch performance of Muscovy ducks.

Key words: Amino acids, In-ovo injection, Muscovy duck, Performance

Introduction

There is a linear improvement in the growth performance and meat yield of commercial poultry with better input efficiency each year (Havenstein et al., 2003; Ferket, 2004). Different factors play crucial roles in influencing hatchability and growth performance during embryonic and post-hatch life, for instance genetic, egg characteristics and incubation environment (Narushin and Romanov, 2002; Petwket et al., 2003; Abiola et al., 2008). Nutrients utilization in the egg's embryo is crucial and their transfer from the mother to her embryo is completed before the egg is laid, thus the egg contains all of nutrients needed for the growth and development of the embryo. Hatched chicks are affected by the nutrients in the yolk remaining in the peritoneal cavity post-hatching (Romanoff, 1960). Nowadays, in ovo-injection is widely used for many purposes, such as fertilizing an avian egg in the shell (Cantrell and Wooten, 2003), injecting avian eggs with immunological material (Jochemsen and Jeurissen, 2002), a trial for sex reversal in birds (Kagmi and Hanada, 1997), increasing the post-hatching body weights of birds by in-ovo injection of growth promoters (Ohta et al., 1999), and enhancing the growth of avian embryo by injecting eggs with special liquid nutritional supplements. Nutrients in-ovo injection has a lot of benefits: greater efficiency of feed utilization (Bhanja et al., 2004); reduced post-hatch mortality and morbidity; improved immune response (Gore and Qureshi, 1997); enhanced early growth by improving intestinal function and development (Tako et al., 2004); and increased skeletal growth (Hargis et al., 1989), breast muscle yield (Hajihosaini and Mottaghitalab, 2004), and marketing body weight (Selim et al., 2012).

Broiler breeder eggs contain an excess of fat and moisture while protein store may be limited (Al-Murrani, 1978), and yolk protein is the origin of the required amino acids (AA) during

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embryogenesis (Gerhartz et al., 1999). Therefore, embryonic and post-embryonic performance may be improved by in-ovo injection of amino acids (Al- Murrani, 1982), and their derivatives either sporadic or in mixture. Amino acids were injected in the fertile eggs of poultry either single amino acid such as arginine and threonine or amino acids metabolites as β-hydroxy-ß-methyl butyrate (a leucine metabolite) or as mixture of essential and/or non-essential amino acids which mostly identical to the amino acids profile of egg protein (pattern relative to lysine). However, researches concerning in-ovo nutrients administration are minimal. Owing to the importance of in-ovo injection and its role in improving the hatchling weight, the objective of this research was to assess the impact of in-ovo injection of two levels of amino acids mixture (0.50 and 0.75 ml) on post-hatch growth, carcass traits, lymphoid organs and antibody titer in Muscovy ducks.

Materials and Methods

Eggs incubation and injection

Standard size fertile duck eggs (n= 500) were collected from Muscovy breeders ducks fed on adequate nutritional diet. Fertility was verified by candling with a hand ultraviolet lamp at day 12 of incubation, Eggs were distributed into five groups of 100 eggs each. Eggs were incubated at 37 to 37.5ºC and 70% relative humidity during the first 32 days of incubation. Eggs were turned automatically every hour until the 32nd day. All eggs were transferred to the hatchery at the end of the 32nd day of incubation, and placed in hatching boxes at 37°C temperature and 70-75 % relative humidity until hatching occurred between days 35 and 36. On the 12th day of incubation, the groups of fertile eggs were distributed into five groups: uninjected control; 0.50 ml amino acids mixture (Aminoleban®; Egypt Otsuka Pharmaceutical Company); 0.50 ml distilled water (D.W); 0.75 ml amino acids mixture; and 0.75 ml distilled water. The treatment solutions or sham control were injected into the yolk of the 12-day-old embryo which was identified by candling with a hand ultraviolet lamp, through a pinhole made at the broad end of the egg, using a 25 mm needle. Prior to in-ovo injection the injection site was disinfected with 70% ethanol and the solutions were warmed to 30°C. The pinhole site was sealed with sterile paraffin wax immediately after injection. The injected eggs were returned to the incubator after injection. This amino acids mixture is of identical pattern to the egg's amino acids, which was calculated as percentage of lysine content of the egg. Where, the ideal ratio of amino acids to lysine remains largely unaffected by dietary, environmental and genetic factors (Schutte and Jong, 2004). The composition of amino acids solution was shown in Table 1.

Ducklings and housing

All hatched ducklings from each treatment were weighed and sexed to males and females subgroups. The ducklings in each treatment group either males or females were randomly assigned to 4 replicates depending upon the hatch size, each replicate with a pen in each treatment. All ducklings were reared under similar managerial and hygienic conditions. The ducklings were raised in clean, well-ventilated, previously disinfected room. A continuous lighting program was maintained throughout the nine weeks experimental period. Temperature was adjusted at 32ºC ± 2 in the first week and then reduced by 2ºC each successive week then maintained at 22ºC ± 2. The ducklings of all groups were vaccinated once against Avian Influenza with 0.50 ml single dose of Reassortant H5N1 Avian Influenza vaccine (Re-1 vaccine) subcutaneously in the lower back of the neck at the 14th day old (Middleton et al., 2007). Ducklings of different experimental groups fed on basal starter (0-3 weeks), grower (3-6 weeks) and finisher (6-9 weeks) rations as shown in Table 2. Basal rations were formulated to meet the nutrients requirements for Muscovy ducks as recommended by French Group Company, Sadat City, Egypt (Strain' origin). Nutrients compositions of the used rations were calculated according to the feed composition tables given by NRC (1994). The ducklings were fed ad libitum on dry mash ration and fresh clean water was constantly available.

Table 1, Composition of injected amino acids* solution into fertile eggs.

L-Amino acids	Content (mg/ml)	% of lysine
Arginine HCL	7.3	96.05
Histidine HCL	3.2	42.10
Methionine	1.0	13.20
Phenylalanine	1.0	13.20
Threonine	4.5	59.20
Valine	8.4	110.50
Lysine HCL	7.6	100.00
Tryptophan	7.0	92.47
Leucine	11.0	144.70
Isoleucine	9.0	118.40
Proline	9.0	118.40
Serine	5.0	66.12
Alanine	7.5	99.16
Cysteine HCL	4.0	52.90

* Identical to egg's amino acids composition.

Growth parameters

After hatching, the body weights (BW) of the ducklings in each group were recorded. The body weight of individual ducklings in each group and feed consumption of each pen were recorded at 3, 6 and 9 weeks of age. The feed conversion ratio (FCR) was calculated accordingly.

Sampling

Blood samples were taken from tibial vein of eight birds in each group (4 males and 4 females) at 5 and 9 week of age by needle under aseptic precaution. Separation of serum was carried out by centrifugation of coagulated blood at 3000 rpm for 15 minutes for determination of antibodies titer against Avian Influenza disease virus by Haemagglutination Inhibition test (Thayer and Beard, 1998). At the end of the experimental period (9 weeks of age) 8 birds (4 males and 4 females) were selected from each treatment group and were slaughtered after being fasted for 12 hours. After slaughter and complete bleeding, the birds were dressed. The carcass and some other components (liver, gizzard, abdominal fat, heart, bursa of Fabricius, spleen, and thymus) were weighed.

Dressing percentage = [(Dressed carcass weight/Live body weight) × 100].

Relative organ weights were calculated as percentages of body weight = [(Organ weight/Body weight) × 100].

Total edible parts were calculated as percentage of body weight = [(Weight of liver + gizzard + heart + abdominal fat)/Body weight) × 100].

Statistical Analysis

The obtained data were presented as means ± SE. Analysis of variance (ANOVA) was used to test the significance of the difference between different treatments and statistical differences were established using a Duncan's Multiple Range Test (Duncan, 1955) at the level of $P < 0.05$.

Results and Discussion

Research has been shown that embryos survived after in-ovo injection of amino acids into the yolk (Al-Murrani, 1982) with little impacts on hatchability percentage (Hajihosaini and Mottaghitalab, 2004; Ohta et al., 1999 and 2001). In-ovo injection of 0.50 ml (84%) but not 0.75 ml of AA mixture (68%) into the yolk of fertilized Muscovy duck's eggs at the 12th day of incubation increased hatchability percentage compared to the un-injected control (74%) and D.W injected groups (72%). However, this was not statistically confirmed. This increase in hatchability percentage after in-ovo injection of 0.50 ml of AA might be due to the timing for injection, during the period of rapid growth of embryo and/or the necessary amount of amino acids administered into fertilized eggs.

Table 2. Composition and nutritional levels of experimental diets.

Ingredients (%)	Ration		
	Starter	Grower	Finisher
Ground yellow corn	61.40	67.00	68.10
Soya bean meal (44 % CP)	30.00	23.40	20.00
Corn gluten meal (60 % CP)	4.30	5.00	5.00
Vegetable oil[1]	0.40	0.70	3.00
Mono calcium phosphate[2]	1.36	1.42	1.36
Ground limestone	1.73	1.72	1.54
Salt (NaCl)	0.31	0.30	0.40
Mineral and Vitamin Premix[3]	0.30	0.30	0.40
L-Lysine[4]	0.10	0.10	0.15
L-Methionine[5]	0.10	0.10	0.10
Calculated composition			
ME MJ/Kg diet	12.2	12.6	13.1
Crude protein (g/kg)	211.0	191.0	177.0
Calcium (g/kg)	9.7	9.6	8.7
Available phosphorus (g/kg)	4.2	4.2	4.0
Lysine (g/kg)	10.8	9.3	8.8
Methionine (g/kg)	4.6	4.4	4.2
Methionine + Cystine (g/kg)	8.1	7.7	7.3

1Vegetable oil composed of a mixture of soybean, cotton seed and sunflower oils.

2Monocalcium phosphate; 21% phosphorus, and 15% calcium.

3Minerals and Vitamins mixture (Pharma Mix). Each 3 kg contain: 12.000.000 I.U. Vitamin A, 2.500.000 I.U. Vitamin D3, 10.000 mg Vitamin E, 2.00 mg Vitamin K3, 1.000 mg Vitamin B1, 5.000 mg Vitamin B2, 1.500 mg Vitamin B6, 10 mg Vitamin B12, 30.000 mg, Niacin 1.000 mg, Folic acid, 50 mg Biotin, 10.000 mg Pantothenic acid, 10.000 mg Copper, 10.000 mg Iodine, 100 mg Selenium, 30.000 mg Iron, 60.000 mg Manganese, 50.000 mg Zinc, 100 mg Cobalt, CaCo3 add to 3000 gm; 4L-lysine: 78% produced by Archar Daniels Medland Company De Caur I.L. made in U.S.A. (ADM); 5DL-methionine: 99% Canadian registration number 990137 Guaranteed analysis, DL-Methionine 99%.

These results are in consistent with Ohta and Kidd (2001) who noted that the percentage of hatchability increased by in-ovo administration of 0.50 ml of AA mixture into the yolk of broiler eggs at the 7th day and at the 14th day of incubation in the work of Bhanja and Mandal (2005). Gaafar (2009) found that in-ovo injection of 0.50 ml of amino acids mixture into the yolk of fertilized Muscovy duck's eggs at day 12th of incubation increased hatchability percentage as compared to the control group. On the other hand, in-ovo injection of nutrients may provide an alternative method to increasing duckling weight at hatch. In-ovo injection of 0.50 ml (55.05 g) or 0.75 ml (52.84 g) of amino acids mixture significantly increased ($P < 0.05$) body weights of ducklings at hatch compared to the uninjected control (45.95 g). On the other hand, there was no significant difference ($P < 0.05$) between the uninjected control and sham groups (47.36 g and 49.45 g for 0.50 ml and 0.75 ml distilled water injected groups, respectively). This increase in ducklings weights might be due to the increased available free amino acids into eggs, that are not normally in excess amount, where the embryo development is restricted to the nutrients content of eggs (Klein, 1968; Rupe and Farmer, 1955) and are excessively utilized during the period from days 7 to 14 of incubation (Ohta and Sato, 2005).

In-ovo injection of identical AA mixture improved post-hatching growth and marketing weight as a result of increasing available AA remaining in the peritoneal cavity post-hatching (Romanoff, 1960), which might be enhanced the protein synthesis after hatching. In-ovo injection of 0.50 or 0.75 ml of AA significantly increased ($P < 0.05$) body weights of males than the un-injected control during the experimental periods. Moreover, there was no significant difference in body weights between in-ovo amino acids females and un-injected control except during the starting period for 0.50 ml and starting and growing periods for 0.75 ml; it significantly increased (Table 3). These results were in agreement with Al-Murrani (1982) who recorded that injection of broiler eggs with 0.50 ml of AA increased body weight by 12.7 % at 56 days of age, and Gaafar (2009) recorded that body weights significantly increased in male and female ducks hatched from eggs injected with amino acids mixture compared with the control group. Foye et al. (2006) reported that in-ovo feeding of 0.7 % Arginine and β-hydroxy-β-methylbutyrate resulted in a 27.5 g higher body weight at 14 days of age. Furthermore, Bhanja and Mandal (2005) reported that in-ovo injection of specific amino acids (Ile + leu + Val) or (Gly + Pro) resulted in 63.2-63.6 g higher body weight at 3rd week of age. In another study, Bakyaraj et al. (2012) found that in ovo feeding of AA for CMI response (lysine, methionine, arginine, leucine, and isoleucine) resulted in significantly higher ($P < 0.01$) body weight at 21 days of age than the sham control group. In the present study, a higher body weight was recorded in the AA groups where arginine, leucine, isoleucine, methionine, lysine, valine and proline were constituents in the in-ovo amino acids mixture solution.

Table 3. Body weight (g) of male and female Muscovy ducklings hatched from eggs injected with two levels of amino acids mixture in different growth periods (mean ± SE).

Group Period	Control	AA (0.50 ml)	D. W (0.50 ml)	AA (0.75 ml)	D. W (0.75 ml)
Male					
Starting	551.6 ± 12.8^{b}	829.7 ± 8.0^{a}	549.4 ± 3.0^{b}	820.0 ± 7.1^{a}	553.0 ± 4.0^{b}
Growing	2196.6 ± 41.2^{c}	2483.6 ± 23.9^{b}	2173.7 ± 10.7^{c}	2608.6 ± 10.5^{a}	2200.0 ± 19.8^{c}
Finishing	4109.2 ± 55.2^{c}	4565.0 ± 31.2^{a}	4055.3 ± 44.0^{c}	4350.0 ± 11.5^{b}	4150.0 ± 14.6^{d}
Female					
Starting	572.0 ± 8.1^{b}	651.0 ± 6.9^{a}	569.2 ± 4.8^{b}	644.7 ± 8.5^{a}	572.8 ± 2.4^{b}
Growing	1770.5 ± 27.7^{b}	1800.1 ± 12.7^{b}	1772.5 ± 11.5^{b}	1916.5 ± 12.0^{a}	1776.7 ± 8.5^{b}
Finishing	2801.9 ± 32.6^{a}	2731.7 ± 56.9^{a}	2765.0 ± 16.1^{a}	2778.1 ± 20.1^{a}	2766.7 ± 15.3^{a}

a-b Means within the same row having different superscript are significantly different ($P < 0.05$). AA = Amino Acids mixture, D.W = Distilled Water

Table 4. Feed conversion ratio (FCR) of male and female Muscovy ducklings hatched from eggs injected with two levels of amino acids mixture in different growth periods (mean ± SE).

Group Period	Control	AA (0.50 ml)	D. W (0.50 ml)	AA (0.75 ml)	D. W (0.75 ml)
Male					
Starting	1.47 ± 0.04[a]	1.49 ± 0.01[a]	1.48 ± 0.02[a]	1.48 ± 0.01[a]	1.47 ± 0.01[a]
Growing	1.82 ± 0.04[a]	1.79 ± 0.02[a]	1.78 ± 0.04[a]	1.78 ± 0.01[a]	1.79 ± 0.04[a]
Finishing	2.21 ± 0.04[a]	2.28 ± 0.03[a]	2.14 ± 0.09[a]	2.25 ± 0.01[a]	2.11 ± 0.05[a]
Female					
Starting	1.40 ± 0.02[a]	1.40 ± 0.01[a]	1.38 ± 0.02[a]	1.36 ± 0.01[a]	1.38 ± 0.01[a]
Growing	2.25 ± 0.04[a]	2.21 ± 0.01[a]	2.23 ± 0.01[a]	2.28 ± 0.03[a]	2.26 ± 0.01[a]
Finishing	3.30 ± 0.06[a]	3.32 ± 0.05[a]	3.38 ± 0.05[a]	3.39 ± 0.07[a]	3.36 ± 0.03[a]

a-b Means within the same row having different superscript are significantly different (P < 0.05). AA = Amino Acids mixture, D.W = Distilled Water

In-ovo injection with 0.50 or 0.75 ml of AA resulted in an increase in the feed intake of male and female ducks as compared to the un-injected control group (data not shown). Corresponding higher body weight was also found in those groups. This higher feed intake of ducklings hatched from eggs injected with amino acids mixture may attribute to greater body weight as feed intake is a function of live body weight wherein all the treatment groups had similar diets. Therefore, the ducklings which had higher body weight must have consumed higher feed, provided there was no difference in FCR (Table 4). These results were in agreement with Bhanja and Mandal (2005) who reported that higher feed intake in the amino acid-injected groups than the control group. Bhanja et al. (2004) recorded that there was no difference in feed intake and FCR, but apparently better FCR was found in AA-injected chicks. In another study, Kadam et al. (2008) reported that feed conversion until 17 day after hatching was improved by in-ovo injection of 10, 20 or 30 mg of threonine. Moreover, Bakyaraj et al. (2012) reported that in ovo injection of AA for CMI response resulted in significantly higher feed intake with no variation in FCR during the 3-week experiment than the untreated control.

There have been substantial studies looking at improving carcass traits. In-ovo injection of nutrients is interested possible method to meet this objective, but there is very little research looking at in-ovo injection with amino acids. In-ovo injection of 0.50 or 0.75 ml of AA caused no significant changes (P < 0.05) in the dressing percentage of male ducks but increased in females group injected with 0.50 ml of AA mixture as compared to un-injected control. On the other hand, In-ovo injection of either 0.50 or 0.75 ml of AA mixture resulted in significant increase (P < 0.05) in the dressing percentage compared to the sham group. In-ovo injection of amino acids mixture resulted in significant increase (P < 0.05) in the liver weight for male and female ducks compared to the un-injected control and sham groups (Table 5).

Table 5. Carcass traits of male and female Muscovy ducks after in-ovo injection with amino acids mixture (mean ± SE):

Group Item(as % of BW)	Control	AA (0.50 ml)	D. W (0.50 ml)	AA (0.75 ml)	D. W (0.75 ml)
Male					
Dressing	82.5 ± 1.1[a]	80.9 ± 0.8[a]	77.0 ± 1.0[b]	82.1 ± 0.2[a]	75.8 ± 0.2[b]
Liver	2.15 ± 0.12[b]	2.47 ± 0.01[a]	2.00 ± 0.01[b]	2.66 ± 0.20[a]	1.85 ± 0.08[b]
Gizzard	2.52 ± 0.08[a]	2.53 ± 0.12[a]	2.24 ± 0.02[a]	2.31 ± 0.06[a]	2.51 ± 0.13[a]
Heart	0.67 ± 0.01[a]	0.65 ± 0.01[a]	0.68 ± 0.01[a]	0.70 ± 0.01[a]	0.69 ± 0.01[a]
Abdominal fat	2.30 ± 0.30[a]	2.03 ± 0.30[a]	2.30 ± 0.40[a]	2.68 ± 0.22[a]	2.30 ± 0.22[a]
Total edible parts	7.64 ± 0.54[a]	7.68 ± 0.16[a]	7.22 ± 0.12[a]	8.35 ± 0.10[a]	7.35 ± 0.16[a]
Female					
Dressing	75.7 ± 0.1[b]	80.1 ± 0.4[a]	72.3 ± 0.4[c]	75.9 ± 1.2[b]	72.6 ± 0.4[c]
Liver	1.92 ± 0.02[c]	2.41 ± 0.01[a]	1.83 ± 0.04[c]	2.09 ± 0.01[b]	1.80 ± 0.03[c]
Gizzard	2.33 ± 0.02[a]	2.23 ± 0.01[a]	2.35 ± 0.03[a]	2.27 ± 0.01[a]	2.35 ± 0.03[a]
Heart	0.77 ± 0.01[a]	0.76 ± 0.01[a]	0.71 ± 0.05[a]	0.78 ± 0.01[a]	0.71 ± 0.04[a]
Abdominal fat	2.64 ± 0.30[a]	2.41 ± 0.40[a]	2.74 ± 0.20[a]	2.37 ± 0.37[a]	2.50 ± 0.36[a]
Total edible parts	7.66 ± 0.12[a]	7.81 ± 0.03[a]	7.81 ± 0.27[a]	7.51 ± 0.05[a]	7.36 ± 0.34[a]

a,b Means within the same row having different superscript are significantly different (P < 0.05). AA = Amino Acids mixture, D.W = Distilled Water

Table 6. Lymphoid organ weight of male and female Muscovy ducks after in-ovo injection with amino acids mixture (Means ± SE).

Group Item	Control	AA (0.50 ml)	D. water (0.50 ml)	AA (0.75 ml)	D. water (0.75 ml)
Male					
Thymus	0.545 ± 0.006^{b}	0.555 ± 0.022^{b}	0.480 ± 0.004^{c}	0.715 ± 0.010^{a}	0.495 ± 0.010^{c}
Bursa	0.131 ± 0.004^{c}	0.145 ± 0.006^{bc}	0.155 ± 0.002^{b}	0.200 ± 0.012^{a}	0.145 ± 0.006^{bc}
Spleen	0.054 ± 0.001^{c}	0.069 ± 0.001^{b}	0.044 ± 0.001^{d}	0.080 ± 0.002^{a}	0.035 ± 0.002^{d}
Female					
Thymus	0.620 ± 0.016^{b}	0.712 ± 0.010^{a}	0.455 ± 0.022^{c}	0.655 ± 0.006^{b}	0.462 ± 0.025^{c}
Bursa	0.118 ± 0.007^{b}	0.200 ± 0.012^{a}	0.135 ± 0.002^{b}	0.132 ± 0.001^{b}	0.128 ± 0.014^{b}
Spleen	0.051 ± 0.001^{b}	0.075 ± 0.001^{a}	0.061 ± 0.003^{ab}	0.060 ± 0.004^{ab}	0.057 ± 0.006^{b}

a,b Means within the same row having different superscript are significantly different ($P < 0.05$). AA = Amino Acids mixture, D.W = Distilled Water

Table 7. Antibody titers (HI titer log – 2)* of male and female Muscovy ducks after in-ovo injection with amino acids mixture (mean ± SE).

Group Item	Control	AA (0.50 ml)	D. W (0.50 ml)	AA (0.75 ml)	D. W (0.75 ml)
Male					
1st antibody titer	3.5 ± 0.2^{a}	3.0 ± 0.4^{a}	3.0 ± 0.2^{a}	3.5 ± 0.2^{a}	3.0 ± 0.3^{a}
2nd antibody titer	3.0 ± 0.4^{a}	3.5 ± 0.2^{a}	3.0 ± 0.4^{a}	3.5 ± 0.2^{a}	3.0 ± 0.4^{a}
Female					
1st antibody titer	3.5 ± 0.2^{a}	3.5 ± 0.2^{a}	3.0 ± 0.2^{a}	3.5 ± 0.2^{a}	3.0 ± 0.2^{a}
2nd antibody titer	3.5 ± 0.2^{a}	3.5 ± 0.2^{a}	3.0 ± 0.5^{a}	4.0 ± 0.4^{a}	3.0 ± 0.6^{a}

a,b Means within the same row having different superscript are significantly different ($P < 0.05$). AA = Amino Acids mixture, D.W = Distilled Water. * Against H5N1 reassortant Avian Influenza vaccine

Total edible parts % of 0.50 or 0.75 ml AA injected males and females were not affected as compared to the control group. These results were nearly similar to the results of Bhanja et al. (2004) who observed that broiler's carcass characteristics and cut-up parts yield at 6 weeks of age did not vary between AA injected and control birds, and Johri (2004) recorded that in-ovo injection of AA did not affect the digestive organs and carcass yield. However, Hajihosaini and Mottaghitalab (2004) found that complete and edible carcass weights were higher in broiler obtained from egg treated with AA injected in yolk sac than in the control group.

The relative weights of thymus gland and bursa of fabricus were comparatively higher in 0.50 ml AA injected males group; however the relative weight of spleen significantly increased ($P < 0.05$) as compared to the control. Moreover, in-ovo injected females had higher lymphoid organ weights ($P < 0.05$) than un-injected control and sham group. On the other hand, in-ovo injection of 0.75 ml of amino acids mixture significantly increased ($P < 0.05$) lymphoid organ weights in male ducks only as compared to the un-injected control and sham group (Table 6). These results were partially agree with Bhanja et al. (2004) who showed that there were no significant differences in the weights of immune organs in the AA injected birds and the control group. However, the weights of thymus and spleen were comparatively higher in AA injected group than the control birds when in-ovo injections were carried out on the 14th day of incubation.

In-ovo injection of fertile eggs with 0.50 or 0.75 ml of AA mixture resulted in no significant changes in the geometric means of the 1st and 2nd estimates for antibodies titers of male and female groups as compared to the control group (Table 7). These results were similar to those of Hajihosaini and Mottaghitalab (2004) who found that no significant difference between the AA injected group and the control one in terms of antibodies titer.

Conclusion

Early supplementation of nutrients through in-ovo injection such as amino acids mixture can be regarded as a possible method to improve hatchability, body weight at hatch, marketing weights, and immune status of Muscovy ducks. Further investigations are needed to highlight the effect of in-ovo injection of amino acids mixture on the humoral, cell mediated immunity, and development of digestive organs in Muscovy ducks.

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