Efficacy of Methionine supplementation on the Growth Performance and Lymphoid organs indices of Broiler Chickens Vaccinated with combined Infectious Bronchitis \ Newcastle disease Vaccines

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Abstract

Methionine (Met.) is amino acid that has proven immune regulatory action. It is usually first limiting amino acid in maize and soybean meal based diet. Met. supplementation in growing chick is a common practice (Swick *et al.,* 1990; Shini & Brydeen, 2005). One of the mechanisms proposed to explain Met. interference in the immune system is the proliferation of T cells, which are sensitive to intracellular glutathione and cysteine levels, compounds also participate in Met. metabolism (Kinscherf *et al.,* 1994). The aim of this study was to investigate the efficacy of Methionine (Met) supplementation on the growth performance and lymphoid organs indices of broiler chickens vaccinated with combined infectious bronchitis (IB)\Newcastle disease (ND) vaccines. A total of 120 newly hatched commercial broiler chicks were assigned into four equal treatment groups as follow: First group chicks were vaccinated against IB and ND at 8 and 21 days of age without Met. Second group chicks were vaccinated against IB and ND at 8 and 21 days of age with 0.5% Met. in the diet. Third group chicks remain as control group. Fourth group chicks remain as control group with 0.5% Met. in the diet. Body weight (BW), body weight gain (BWG), feed intake (FI) and lymphoid organs indices were estimated in all groups. Statistical analysis revealed significant ($P<0.05$) differences among the treatment groups in BW, BWG and better FI as compared with non treatment group. The mean value of thymus, bursa of Fabricius and spleen index of chicks supplemented Met during the period of age showed significant increment ($P<0.05$). We concluded that better growth performance and lymphoid indices could be obtained with adequate supplementation of Met which have been identified to be in marginal quantities in poultry feed.

Keywords: **Methionine; growth performance; lymphoid organs indices; broiler.**

Introduction

The importance of poultry production has increased during recent decades and will increase in the future due to the rise in demand for food, together with human population growth. In this context, infectious diseases are important in broiler industry due to increased mortality, growth retardation, and the curative and preventive use of antibiotics and chemotherapeutics (Goren, 1991; Vandemaele *et al.,* 2002; McKissick, 2006).

Met is the first limiting essential *amino acid* (Gill, 2003) in most of plant protein sources (corn and soybean meal), used in typical chicken diets and a supplement is essential for growth and egg production for poultry (Ravindran & Bryden, 1999). Thus requirements in Met and total Sulphur *amino acid* have been

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largely addressed including immunity in addition to growth (Corzo *et al*., 2005).

One essential function of Met in neurulation may be as precursor for S-adenosylmethionine, the methyl donor in transmethylation reactions (Graat *et al.,* 1996), which plays a main role as the biological methyl donor for the methyla- tion of DNA, RNA, and protein (Kano *et al.*, 1982). A deficiency of Met depresses both the amount of food consumed and body weight gain and Met deficiency is a cause of nutritional myopathy (Cummins *et al.,* 1985). Moreover Met is a protective factor against various types of liver damage (Mori & Hirayama, 2000; Feo *et al.,* 1986).

The IB and ND are an important viral diseases result in considerable economic losses to the poultry industry worldwide (Cavanagh, 2003), most characterized by respiratory signs, poor weight gain, reduced feed efficiency, a drop in egg production and quality and mortality (Kapczynski *et al.*, 2002).

The potential for interference between corona/virus (IBV) and paramyxovirus (NDV) exists given that both of these RNA viruses initially infect epithelial cells of the respiratory tract of the chicken and replicate in the cell's cytoplasm, Since the 1950, the efficacy of combination vaccines, viruses given separately has been studied, The replication of IBV has been shown to interfere with NDV infection (Gelb, 2003). Therefore, there is much evidence that Met improve immunity of the birds against different diseases (Eduardo *et al.,* 2009).

The purposes of this study were to determine the efficacy of Met supplementation on the growth performance and lymphoid indices of broiler chickens vaccinated with combined IB\ND vaccines to assess the BW, BWG, FI and thymus, bursa, spleen indices were employed.

Materials and Methods

Birds and Experimental Design

120 newly hatched commercial broiler chicks “Ross strain” was purchased from a local hatchery**.** Upon arrival, the chicks were weighed and divided randomly into four equal groups including two treated groups. First and second groups were vaccinated by combined of live attenuated IBV vaccine Ma5 strain (Intervet, Holland) and live attenuated NDV vaccine LaSota strain (Intervet– Holland) at days 8 and 21of age as

first and second vaccination without and with 0.5% Met (Evonik Degussa, Belgium) respectively. Third and Fourth groups chicks remain without vaccination but without and with 0.5% Met respectively as control groups. The chickens were raised according to routine management practice. All nutrients including water were supplied *ad libitum*.

Body Weight performance

The chickens were weighed individually weekly to determine BW and BWG. The FI was measured for the feeding periods of starter (day 0-21), and finisher (day 22-35) and cumulatively (day 0-35).

Lymphoid organs indices

Birds from each group were sacrificed by slaughtering at the stipulated period, i.e at days 18 and 31. Following a thorough visual appraisal, the, thymus, bursa and spleen, were immediately removed, stripped of fat and connective tissue, blotted dry and individually weighed. Since substantial organ weight change was anticipated, their indices were calculated (Sellers *et al.*, 2007; Williams & Zedek, 2010).
Organ index = organ weight (g)/BW (g) x 100

Statistical Analysis

The data were analyzed using one-way analysis of variance. Differences between means were determined using Turkey's tests in which the significance level was designated at ($P<0.05$).

Results and Discussion

Body Weight performance

The results of body weight of broilers during the experimental period are shown in Table 1. An Increase in body weight values was seen in all groups during the course of the experiment. No significant ($P > 0.05$) group difference was detected for mean body weight at day 7. From day 7 to the end of the experiment day 35, the Second and Fourth (Met) groups had the highest ($P < 0.05$) mean body weight values.

Table (1): The effect of Met supplementation on body weight (g) of broilers during the experimental period (mean±SD)

Groups	Day1	Day 7	Day 14	Day 21	Day 28	Day 35
IB+ND	47.8 ± 3.3^{a}	219 ± 4.6^{a}	449 ± 16.4^{b}	810 ± 6.8^{b}	1511 ± 38.4^{b}	1938 ± 43^{b}
IB+ND+Met	48.2 ± 3.1^{a}	210 ± 16.3^{a}	492 ± 25.9^{a}	859 ± 9.5^{a}	1588 ± 21.8^{a}	1977 ± 56^{ab}
Control	49.6 ± 1.1^{a}	207 ± 4.6^{a}	460 ± 10.3^{b}	800 ± 29.3^{b}	1530 ± 18.9^{b}	1990 ± 37^{ab}
Control+Met	48.4 ± 2.7^{a}	212 ± 8.3^{a}	490 ± 15.9^{a}	869 ± 21.5^{a}	1598 ± 29.8^{a}	2060 ± 61^{a}

[a, b] Values bearing similar superscript in the same column do not differ at ($P < 0.05$).

Similarly, an increasing pattern of body weight gain was seen in all groups as time advances (Table 2). However, such increment of body weight gain in the second and fourth (Met) groups were the fastest and highest values in compared with the vaccinated (IB+ND and control) groups.

Table (2): The effect of Met **supplementation on body weight gain (g) of broilers during the experimental period (mean±SD)**

Groups	1-21 d	21-35 d	1-35 d
IB+ND	764 ± 7.9[b]	1125 ± 12.4[a]	1880 ±33.9[b]
IB+ND+Met	810 ± 7.2[a]	1127 ± 50.9[ab]	1930 ±60.4[ab]
Control	750 ± 29.4[b]	1182 ± 31.9[a]	1930 ±44.6[b]
Control+Met	820 ± 21.7[a]	1195 ± 35.7[a]	2007 ±35.9[a]

[a, b] Values bearing similar superscript in the same column do not differ at ($P < 0.05$).

The FI consistently increased as a time advanced in all the groups throughout the trial (Table 3). Nevertheless, manifested fluctuations for FI in (Met and non-Met) groups during the course of the experiment without being subjected to statistical test due to single sampling.

Table (3): Weekly feed intakes (g) of broilers during the experimental period.

Groups	0-7	7-14	14-21	21-28	28-35
IB+ND	185	463	631	773	875
IB+ND+Met	170	450	603	764	840
Control	173	439	687	828	860
Control+Met	175	405	650	800	833

These result of growth performance showed that the deficient levels of essential amino acids had a negative effect on the growth performance in broilers. This effect was most pronounced for Met. or sulphur containing amino acids as they were regarded as first limiting. As to the main factors, it is possible to observe that the non-vaccinated bird group had better BW, BWG, and FI, indicating that stress resulted from vaccinal virus replication may affect body weight performance during vaccination period as observed in other vaccinal infections, such as those with infectious bursal disease virus vaccine strains (Michell *et al*., 2009).

The deleterious effects of single essential amino acid deficiencies on growth and feed intake vary depending on the specific amino acid

involved (Kino & Okumura, 1986). However, improvements in feed utilization as a result of Met supplemen- tation have been widely observed in broiler chickens, and increases in Met levels promoted an increase of approximately 12 to 14% in BW gain compared with broilers receiving a Met deficient diet (Meirelles *et al.*, 2003; Wang *et al.*, 2004).

Improvement in feed conversion ratio in Met groups, represents a more feed efficiency due to enhanced performance in metabolism of energy and protein which is in agreement with some studies (Zulkifli *et al*., 1994; Si *et al.*, 2001).

Lymphoid organs indices

The somatic index of thymus, bursa and spleen of chickens during the experiment are shown in Tables 4 and 5. Although an increased in somatic index was seen in Met supplementation groups, the indices values remained comparable between all groups, at days 18 and 31. All groups registered a decreasing trend of thymus, bursa and spleen somatic index over time.

Table (4): The effect of Met **supplementation on lymphoid organ indices of broilers during the experimental period (mean±SD)**

Groups	Thymus index d 18	Bursa index d 18	Spleen index d 18
IB+ND	0.490 ± 0.016^{b}	0.240 ± 0.013^{b}	0.091 ± 0.008^{b}
IB+ND+Met	0.564 ± 0.028^{a}	0.266 ± 0.018^{a}	0.108 ± 0.008^{a}
Control	0.530 ± 0.054^{ab}	0.195 ± 0.010^{c}	0.104 ± 0.002^{ab}
Control+Met	0.575 ± 0.082^{a}	0.201 ± 0.008^{c}	0.104 ± 0.009^{ab}

$^{a, b, c}$ Values bearing similar superscript in the same column do not differ at ($P < 0.05$).

Table (5): The effect of MET supplementation on lymphoid organ indices of broilers during the experimental period (mean±SD)

Groups	Thymus index d 31	Bursa index d 31	Spleen index d 31
IB+ND	0.211 ± 0.010^{ab}	0.084 ± 0.007^{b}	$0.065\pm.005^{ab}$
IB+ND+Met	0.243 ± 0.026^{a}	0.107 ± 0.009^{a}	$0.073\pm.006^{a}$
Control	0.190 ± 0.011^{b}	0.070 ± 0.003^{c}	$0.060\pm.003^{b}$
Control+Met	0.198 ± 0.012^{b}	0.073 ± 0.004^{bc}	$0.063\pm.004^{ab}$

[a, b, c] Values bearing similar superscript in the same column do not differ at ($P < 0.05$).

The immune or lymphatic organs, including the central and peripheral lymphoid organs, can produce and cultivate lymphocytes. The central lymphoid organs of birds, namely the thymus and cloacal bursa, exist to generate, differentiate, and mature T and B lymphocytes, respectively. The central lymphoid organs maintain normal immunological function and enhance resistance against disease. The spleen is an important peripheral lymphoid organ, and is the location of immune response production (Hui SONG *et al.*, 2012)

Our results were in the same line who stated that nutrient deficiencies were particularly deleterious to the immune system Zulkifli *et al.*, (1994) when they occured early in life during the development of the primary lymphoid organs and the maturation of immune system. Fasuyl & Aletor, (2005) reported that better performance can still be obtained with adequate supplementation of essential amino acids especially Met. which has been identified to be in marginal quantities in most poultry feeds. However, the lymphoid organ weights increment might be due to the general improvement of the body weight of birds supplemented with Met. which might cause lymphocytes repletion of lymphoid organs resulting in greater thymus, splenic and bursal weights and indices. Dietary Met. deficiency could cause the mal-development of lymphoid organs and their normal function (Konashi *et al.*, 2000; Carew *et al.*, 2003).

In conclusion adequate Met supplementation was a little bit enhanced the body weight performance and lymphoid organs indices in broilers. Additional studies are needed to assess the exact mechanism exerted by Met on broiler growth performance and lymphoid organs indices.

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فاعلية اضافة المثيونين على اداء النمو ومؤشرات الاعضاء اللمفاوية في دجاج اللحم الملقح معا بلقاحي التهاب القصبات المعدي والنيوكاسل

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الخلاصة

ميثيونين حوامض أمينية التي يعمل في تنظيم للمناعة. هي حوامض أمينية محددة أولية عادة موجودة في الذرة الصفراء والصويا كوجبة طعام في العليقة. أضافة الميثيونين في عليقة الافراخ النامية شائع. أحدى الأليات المقترحة لتوضيح تدخل الميثيونين في نظام المناعة هو انتشار خلايا T، الذي حساس لمستويات الكليوتاثيون والسيستين، تشارك المركبات أيضا في أيض الميثيونين. ان هذه الدراسة تهدف إلى التعرف على فعالية اضافة الميثيونين على أداء النمو ومعدل وزن غدة التوثة، جراب فابريشا والطحال إلى وزن الجسم في الدجاج اللاحم الملقحة بلقاحي التهاب الشعب الهوائية المعدية ونيوكاسل. استعمل في هذه الدراسة 120 من افراخ الدجاج اللاحم التجارية بعمر يوم واحد قسمت هذه الافراخ إلى أربع مجموعات متساوية على النحو التالي في المعاملة: المجموعة الاولى (G1) تم تحصين الافراخ ضد التهاب الشعب الهوائية المعدية ونيوكاسل في 8 و 21 يوما من العمر دون اضافة الميثيونين. المجموعة الثانية (G2) تم تحصين الافراخ ضد التهاب الشعب الهوائية المعدية ونيوكاسل في 8 و 21 يوما من العمر مع إضافة 0.5٪ من الميثيونين الى العليقة. المجموعة الثالثة (G3) عدت كمجموعة سيطرة. اما المجموعة الرابعة (G4) عدت كمجموعة سيطرة ولكن باضافة الميثيونين 0.5٪ الى العليقة.

تم جمع البيانات من زيادة الوزن، الزيادة في وزن الجسم واستهلاك العلف من عمر (0-35) يوم. وكشف التحليل الإحصائي للبيانات وجود فروق معنوية ($P<0.05$) بين مجموعات المعالجة في الوزن وبالزيادة الوزنية. على اية حال، اظهر النتائج وجود زيادة بالوزن وبالزيادة الوزنية وزيادة استهلاك العلف في المجموعتين المضافة اليها المثيونين. وأظهر زيادة معدل قيم غدة التوثة، جراب فابريشا والطحال في الافراخ المضافة اليها الميثيونين خلال فترة التربية فروق معنوية ($P <0.05$). خلصنا إلى أن أفضل أداء النمو ومؤشرات اللمفاوية يمكن الحصول عليها مع اضافة كافية من المثيونين التي تم تحديدها لتكون في الكميات الهامشية في أعلاف الدواجن