JAPR

***Vol. 02, Issue, 01, pp. 10-15, 9 May*, 2025**

RESEARCH ARTICLE

Effect of Potenmic Supplementation on Immunity, Blood Metabolites, and Antioxidants Status In Broiler Chicks Fed A Low Crude Protein-Metabolizable Energy Diet

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ARTICLE INFO

Article History:
Received 5 *April*, 2025
Received in revised form 23 *April*, 2025
Accepted *3 May, 2025*
Published online *9 May, 2025*

Keywords:
Antioxidant status
Blood metabolites
Broiler chicks
Immune response
Potenmic

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ABSTRACT

This study evaluated the effects of Potenmic supplementation on immunity, blood metabolites, and antioxidant status in broiler chicks fed a low crude protein (CP) and metabolizable energy (ME) diet (19% CP, 2900 kcal/kg ME). A positive control (PC) diet (22% CP, 3000 kcal/kg ME) and a negative control (NC) diet were compared with NC supplemented with Potenmic at 0.20, 0.33, and 0.67 mL/L of drinking water during starter and finisher phases. The NC diet reduced antibody titers against Newcastle disease (ND), while Potenmic at 0.33 mL/L significantly improved ND immunity in both phases. For infectious bronchitis (IB), 0.20 mL/L was sufficient to enhance antibody response. The heterophil-to-lymphocyte (H:L) ratio, a stress indicator, decreased with Potenmic supplementation, showing a significant reduction of 0.18 points at 0.33 mL/L in the finisher phase. Blood glucose levels increased by 43.70 mg/dL at the highest Potenmic dose (0.67 mL/L) but stabilized at lower concentrations. Serum protein levels improved across all Potenmic doses. Antioxidant status, assessed through malondialdehyde (MDA) levels, indicated reduced oxidative stress at 0.67 mL/L (starter) and 0.33 mL/L (finisher). In conclusion, Potenmic supplementation at 0.67 mL/L (starter) and 0.33 mL/L (finisher) in low CP-ME diets enhances immune response, optimizes blood metabolites, and improves antioxidant status in broiler chicks.

1. Introduction

Agriculture is a vital sector of Pakistan's economy, contributing over 23 % to the GDP and employing approximately 37.4 4 % of the labor force [1]. Around 63% of the country's population resides in rural areas and relies directly or indirectly on agriculture for their livelihood [2]. With Pakistan's population growing at an annual rate of 2.4% [1], the demand for agricultural products, including high-quality protein sources, continues to rise. Livestock, a critical sub-sector of agriculture, contributes 56.3% to agricultural value addition and 11% to the national GDP, while supporting over 36 million people [3]. This sector plays a key role in bridging the gap between animal protein demand and availability [4]. Within livestock, the poultry industry is particularly significant, contributing 1.3% to the GDP [5] and serving as an efficient source of high-quality protein. Poultry meat and eggs provide superior protein digestibility-corrected amino acid scores (PDCAAS) compared to plant-based proteins [6], making them essential for human nutrition.

Historically, antibiotics have been widely used in poultry production to enhance growth performance, improve feed efficiency, and prevent diseases [7]. However, their overuse has led to antibiotic resistance, posing a significant public health risk [8]. In response, the European Union has imposed strict regulations on antibiotic use (Directive 96/23/EC), prompting the search for natural alternatives such as phytogenic feed additives, probiotics, and prebiotics [9,10]. These alternatives not only improve gut health and immunity but also enhance meat quality [11,12]. Potenmic, a commercially available nutritional supplement, contains a blend of amino acids, vitamins, minerals, and trace elements. It acts as an immune booster, antioxidant, metabolic enhancer, and growth promoter in poultry. Previous studies suggest that Potenmic supplementation can improve feed utilization, weight gain, and overall broiler performance [13]. However, limited research exists on its effects on immunity, blood metabolites, antioxidant status, and nutrient digestibility in broilers fed low crude protein (CP) and low metabolizable energy (AME) diets.

Given the growing need for sustainable poultry production and the reduction of antibiotic use, this study aims to evaluate the efficacy of Potenmic supplementation in enhancing immunity, blood biochemical parameters and antioxidant capacity in broiler chicks fed a low CP-AME diet. The findings will provide valuable insights into optimizing broiler nutrition while minimizing reliance on conventional growth promoters.

2. Materials and methods

2.1. Experimental Design and Bird Management

The research was carried out at the University of Agriculture, Peshawar's poultry facility in Pakistan. A group of 150 one-day-old broiler chicks were acclimatized for five days before being randomly assigned to five experimental groups, each containing 30 birds (10 birds per replicate). Potenmic, a commercial supplement containing multivitamins, amino acids, minerals, and trace elements, was administered through drinking water. Birds were housed in an open-sided poultry shed with continuous access to feed and water. Standard vaccination, ventilation, and management practices were followed throughout the trial. The 35-day study was divided into two phases: the starter phase (0–21 days) and the finisher phase (22–35 days).

Treatments Description	Replicates (R1, R2, R3)		
Positive control (PC) – 22% CP, 3000 kcal/kg AME	10	10	10
Negative control (NC) – 19% CP, 2900 kcal/kg AME	10	10	10
NC + 1 ml Potenmic per 1.5 L water	10	10	10
NC + 1 ml Potenmic per 3.0 L water	10	10	10
NC + 1 ml Potenmic per 5 L water	10	10	10

2.2 Blood Collection and Analysis

Blood samples were collected from two birds per replicate at 21 and 35 days via wing vein puncture. Serum was separated by centrifugation (3000 rpm, 10 min) and stored at −20°C for analysis.

2.3 Antioxidant Status

Malondialdehyde (MDA) levels (lipid peroxidation marker) were measured spectrophotometrically at 352 nm using the thiobarbituric acid reactive substances (TBARS) method [14].

2.4 Immune Status

Antibody titers against Newcastle Disease (ND) and Infectious Bronchitis (IB) were assessed via hemagglutination inhibition (HI) test [15]. Heterophil-to-lymphocyte (H:L) ratio was determined using Giemsa-stained blood smears [16].

2.5 Blood Metabolites:

Plasma glucose and total protein concentrations were analyzed using commercial kits (MTD Diagnostics, Italy) via spectrophotometry (546 nm).

2.6 Statistical Analysis

Data were analyzed using ANOVA (SAS, 1998) with LSD post hoc tests ($p < 0.05$). Results are presented as mean ± SEM.

3 Results

3.1 Antibody Titer Against Newcastle Disease (ND)

The supplementation of Potenmic significantly improved antibody titers against Newcastle disease in broiler chicks during both the starter (day 21) and finisher (day 35) phases ($P < 0.01$). At day 21, the negative control (NC) group, fed a low-protein and low-energy diet (19% CP, 2900 kcal/kg ME), exhibited the lowest antibody titer (3.00), which was significantly lower than the positive control (PC) group (4.33). However, supplementation with Potenmic at 1 mL/1.5 L of drinking water (NCPot1) dramatically increased the antibody titer to 7.33, demonstrating a substantial immune enhancement. Reducing the Potenmic concentration to 1 mL/3 L (NCPot2) resulted in a slight decline (6.66), while further dilution to 1 mL/5 L (NCPot3) led to a more noticeable reduction (5.66). By day 35, a similar trend was observed, with NCPot1 maintaining the highest titer (6.33), followed by NCPot2 (5.00) and NCPot3 (4.66). These findings indicate that Potenmic, particularly at 1 mL/1.5 L, effectively enhances humoral immunity against ND, even in birds fed a suboptimal diet.

3.2 Antibody Titer Against Infectious Bronchitis (IB)

Potenmic supplementation also significantly influenced antibody production against Infectious bronchitis (P < 0.05). At day 21, the highest antibody titer was observed in the NCPot2 group (5.66), followed by NCPot3 (5.33) and NCPot1 (5.00), all of which were significantly higher than the NC (4.00) and PC (3.33) groups. By day 35, NCPot2 continued to exhibit the strongest immune response (6.33), with NCPot3 (5.66) and NCPot1 (5.33) also showing elevated titers compared to the control groups. Interestingly, while Potenmic at 1 mL/1.5 L (NCPot1) was most effective for ND, the 1 mL/3 L dose (NCPot2) elicited the best response against IB, suggesting that the optimal dosage may vary depending on the pathogen.

3.2 Heterophil-to-Lymphocyte (H:L) Ratio

The H:L ratio, a key indicator of stress, was significantly influenced by Potenmic supplementation (P < 0.05). At day 21, the lowest ratio was observed in the NCPot3 group (0.37), while the highest was in NCPot2 (0.60) and PC (0.57). By day 35, NCPot2 (0.33) and NCPot1 (0.39) exhibited the most favorable (lowest) ratios, indicating reduced stress levels, whereas the PC (0.68) and NC (0.51) groups showed higher stress responses. These results suggest that Potenmic, particularly at intermediate concentrations (1 mL/1.5–3 L), helps mitigate physiological stress in broilers, contributing to improved overall health.

3.3 Plasma Glucose Concentration

Potenmic supplementation significantly affected plasma glucose levels (P < 0.01). At day 21, the NCPot1 group had the highest glucose concentration (169.70 mg/dL), followed by NCPot2 (150.44 mg/dL) and PC (145.37 mg/dL), while NC (126.00 mg/dL) and NCPot3 (117.75 mg/dL) had the lowest levels. By day 35, NCPot2 (169.12 mg/dL) and NCPot1 (166.37 mg/dL) maintained elevated glucose levels, whereas PC (122.83 mg/dL) and NC (119.33 mg/dL) remained significantly lower. These findings indicate that Potenmic enhances glucose metabolism, potentially improving energy availability in birds fed low-protein diets.

Table 2: Efficiency of potenmic on antibody titer against new castle disease on broiler chicks at day 21 and 35

Dietary Groups	Newcastle Disease Antibody Titers	
	Day-21	Day-35
PC (Positive Control)	4.33 ± 0.33^{c}	4.00 ± 0.57^{bc}
NC (Negative Control)	3.00 ± 0.00^{d}	3.00 ± 0.0.57^{c}
NCPot1 (Potenmic Dose 1)	7.33 ± 0.33^{a}	6.33 ± 0. 0.33^{a}
NCPot2 (Potenmic Dose 2)	6.66 ± 0.333^{a}	5.00 ± 0.0.57^{ab}
NCPot3 (Potenmic Dose 3)	5.66 ± 0.33^{b}	4.66 ± 0.3^{b}

Significant differences (P < 0.01) were observed across groups at both time points.
Means within a column followed by distinct superscript letters (^{a}, ^{b}, ^{c}, ^{d}) differ significantly (P < 0.05).
[1] PC and NC stand for the positive control and negative control, respectively while the NC_{Pot1}, NC_{Pot2} and NC_{Pot3} stand for the negative control enriched with *Potenmic* @ 1ml per 1.5, 3 and 5 liter of water respectively.

Table 3. Effect of Potenmic supplementation on infectious bronchitis antibody levels in broiler chickens during grower and finisher phases

Experimental Diets[1]	Infectious bronchitis Antibody titer	
	Day-21	Day-35
Positive Control (PC)	3.33 ± 0.33^{c}	3.33 ± 0.33^{b}
Negative Control (NC)	4.00 ± 0.57^{bc}	4.00 ± 0.57^{b}
NC + Potenmic (1ml/1.5L)	5.00 ± 0.33^{ab}	5.33 ± 0.0.33^{a}
NC + Potenmic (1ml/3L)	5.66 ± 0.33^{a}	6.33 ± 0.0.69^{a}
NC + Potenmic (1ml/5L)	5.33 ± 0.33^{ab}	5.66 ± 0.69^{a}

Significant variations (P = 0.02 at day 21; P < 0.01 at day 35) were observed among treatment groups. Values within columns bearing different superscript letters (^{a}, ^{b}, ^{c}) differ significantly (P < 0.05)
Treatment Details:
PC: Basal diet (positive control)
NC: Non-supplemented diet (negative control)
NCPot1-3: Negative control diet supplemented with Potenmic at concentrations of 1ml per 1.5, 3, and 5 liters of drinking water, respectively

Table 4. Influence of Potenmic supplementation on heterophil-to-lymphocyte ratio in broiler chickens during growth phases

Experimental Groups	Hetrophils:lymphocytes	
	Day 21	Day 35
Positive Control (PC)	0.57±0.080[ab]	0.68±0.070[a]
Negative Control (NC)	0.44±0.040[ab]	0.51±0.020[ab]
NC + Potenmic (1ml/1.5L)	0.47±0.030[ab]	0.39±0.070[bc]
NC + Potenmic (1ml/3L)	0.60±0.080[a]	0.33±0.050[c]
NC + Potenmic (1ml/5L)	0.37±0.080[b]	0.38±0.020[bc]
P-Value	0.21	< 0.01

Means within the column with different superscripts are significantly different at probability level of 0.05.
[1] PC and NC stand for the positive control and negative control, respectively while the NC_{Pot1}, NC_{Pot2} and NC_{Pot3} stand for the negative control enriched with *Potenmic* @ 1ml per 1.5, 3 and 5 liter of water respectively.

3.4 Plasma Protein Concentration

Potenmic supplementation also significantly increased plasma protein levels (P < 0.01). At day 21, NCPot1 (5.48 g/dL) and NCPot2 (5.47 g/dL) exhibited the highest protein concentrations, while PC (4.65 g/dL) and NC (4.91 g/dL) were significantly lower. By day 35, NCPot1 (6.70 g/dL) showed the most pronounced improvement, followed by NCPot2 (6.11 g/dL), whereas PC (4.23 g/dL) and NC (4.08 g/dL) remained at baseline levels. This suggests that Potenmic enhances protein synthesis or retention, counteracting the negative effects of a low-protein diet.

3.5 Malondialdehyde (MDA) Levels (Antioxidant Status)

Potenmic supplementation significantly reduced MDA levels, a marker of oxidative stress (P < 0.01). At day 21, the lowest MDA levels were observed in NCPot1 (2.30 nmol/mL), followed by NCPot2 (2.50 nmol/mL) and NCPot3 (2.51 nmol/mL), all of which were significantly lower than PC (2.89 nmol/mL) and NC (2.85 nmol/mL). By day 35, NCPot1 (2.14 nmol/mL) continued to exhibit the strongest antioxidant effect, with NCPot2 (2.26 nmol/mL) also performing well, while PC (2.93 nmol/mL) and NC (2.80 nmol/mL) showed higher oxidative stress. These results demonstrate that Potenmic, particularly at 1 mL/1.5 L, enhances antioxidant capacity, protecting broilers from oxidative damage.

Table 5. Effects of Potenmic supplementation on blood glucose levels in broiler chickens during growth phases

Treatment Groups	Plasma Glucose Concentration (mg / dL).	
	Day-21	Day-35
Positive Control	145.370±1.750[b]	122.830±2.400[b]
Negative Control	126.000±7.570[c]	119.330± 8.410[b]
Potenmic 1.5L	169.700±8.750[a]	166.370± 2.700[a]
Potenmic 3L	150.440±1.830[b]	169.120±1.050[a]
Potenmic 5L	117.750±1.740[c]	132.050±6.340[b]

Statistical Analysis:
Significant treatment effects observed at both time points (P < 0.01)
Values within columns marked with different superscript letters ([a], [b], [c]) differ significantly (P < 0.05)
Treatment Details:
Positive Control: Standard diet with performance enhancers
Negative Control: Basal diet without supplements
Potenmic Groups: Negative control diet supplemented with Potenmic at: 1ml/1.5L water (Potenmic 1.5L); 1ml/3L water (Potenmic 3L) 1ml/5L water (Potenmic 5L)

Table 6. Effects of Potenmic supplementation on plasma protein levels in broiler chickens during growth phases

Experimental Groups	Plasma Protein conc; (g / dl).	
	Day 21[st]	**Day 35[th]**
PC	4.65±0.08[b]	4.23±0.06[c]
NC	4.91±0.15[b]	4.08± 0.46[c]
NC_{Pot1}	5.48±0.20[a]	6.70± 0.04[a]
NC_{Pot2}	5.47±0.15[a]	6.11±0.01[a]
NC_{Pot3}	5.38±0.08[a]	5.07±0.03[b]
P-Value	< 0.01	< 0.01

Means within the column with different superscript are significantly different at probability level of 0.05

Table 7. Impact of Potenmic supplementation on lipid peroxidation levels in broiler chickens measured through malondialdehyde (MDA) concentration

Treatment Groups	Malondialdehyde (MDA; nmol/mL)	
	Day-21st	Day-35th
Positive Control (PC)	2.890±0.05[a]	2.930±0.03[a]
Negative Control (NC)	2.850±0.04[a]	2.800±0.08[a]
NC + Potenmic (1mL/1.5L)	2.300±0.03[c]	2.140±0.05[c]
NC + Potenmic (1mL/3L)	2.500±0.02[b]	2.260±0. 0.07[c]
NC + Potenmic (1mL/5L)	2.510±0.01[b]	2.540±0.05[b]

Statistical Analysis: Significant treatment effects observed at both sampling periods (P < 0.01)**.** Values within columns marked with different superscript letters ([a], [b], [c]) differ significantly (P < 0.05)

4 Discussion

The present study evaluated the effects of Potenmic supplementation on immunity, blood metabolites, and antioxidant status in broiler chicks fed a low crude protein-metabolizable energy (CP-ME) diet. The findings demonstrated significant improvements in heterophil-to-lymphocyte (H:L) ratio, antioxidant status, plasma glucose, protein levels, and antibody titers against infectious bronchitis (IB) and Newcastle disease (ND).

4.1 Heterophil-to-Lymphocyte (H:L) Ratio

The H:L ratio, a key stress and immune status indicator [16], was significantly lower (P < 0.05) in NCPot3 (grower phase) and NCPot2 (finisher phase). A reduced H:L ratio signifies better immune function and lower stress levels [17; 18]. This improvement can be attributed to zinc sulphate, lysine, and methionine in Potenmic, which modulate immune responses. Our results align with Chitithoti et al. (2012), who reported that higher zinc supplementation reduced the H:L ratio, and Mehrdad et al. [18], who observed similar effects with methionine and lysine supplementation.

4.2 Antioxidant Status

Potenmic significantly (P < 0.05) reduced serum malondialdehyde (MDA) levels, particularly in NCPot1, indicating enhanced antioxidant capacity. This effect is likely due to vitamin E, zinc, and selenium, which inhibit lipid peroxidation [20, 21]. Trace minerals like Mn, Cu, Zn, and Se further suppress free radicals, protecting immune cells [22]. Our findings corroborate Kazim et al. (2003), who noted decreased MDA with increased zinc, and K. Sahin et al. [23], who reported similar effects with vitamin E.

4.3 Plasma Glucose and Protein

Potenmic supplementation led to significantly (P < 0.05) higher plasma glucose in NCPot1 (grower) and NCPot3 (finisher), likely due to its glucose and fructose content. These results agree with Shapiro et al. [24] and Cho et al. [25], who observed elevated glucose levels with hyperglycemic diets. However, Kaya et al. [26] reported no effect of zinc on glucose, suggesting other Potenmic components may play a role. For plasma protein, NCPot1 showed the highest levels (P < 0.05), possibly due to essential amino acids and antioxidants (vitamin C & E) in Potenmic. This aligns with Shapiro et al. [24], who found high-protein diets with methionine and lysine increased serum protein, and Sahin et al. [27], who reported similar effects with vitamin E and zinc.

4.4 Antibody Titer Against IB and ND

Potenmic significantly (P < 0.05) enhanced antibody titers against IB (NCPot2) and ND (NCPot1). Zinc likely stimulated T-helper cells, promoting B-lymphocyte activation [28], while selenium and vitamin E further boosted immune responses. These findings are consistent with Hudson et al. [29], who reported higher NDV antibody titers with zinc, and Lin and Chang [30], who noted improved immunity with selenium and vitamin E.

5. Conclusion

Potenmic supplementation in low CP-ME diets enhances immunity, reduces oxidative stress, and improves metabolic health in broilers. Its effects on H:L ratio, antioxidant status, glucose, protein, and antibody production highlight its potential as a functional feed additive for poultry nutrition.

Acknowledgments

The present work is supported by the Department of Animal Nutrition, and College of Veterinary Sciences, The University of Agriculture, Peshawar.

Financial support and sponsorship
The study was not received any internal or external funds.

Conflicts of interest
There are no conflicts of interest.

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