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Research Article

Synergistic Effects of Zinc-DETA, Zinc-Fulvic Acid, and Soya Lecithin on Growth Performance, Immunity and Antioxidant Status in Broiler Chickens: A Comparative Study

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Abstract

This study evaluated the comparative effects of different zinc sources and combinations, including Zinc Sulfate ($ZnSO_4$), Zinc-DETA complex, Zinc-Fulvic Acid complex, and their synergistic combination with and without soya lecithin, on the growth performance, immune response, antioxidant status, and zinc retention in broilers. A total of 300 Cobb 430 broiler chicks were divided into five treatment groups: T1 ($ZnSO_4$), T2 (Zn-DETA), T3 (Zn-Fulvic Acid), T4 (Zn-DETA + Zn-Fulvic Acid), and T5 (Zn-DETA + Zn-Fulvic Acid + Soya Lecithin). Over a 42-day trial, Parameters assessed included body weight gain, Feed Conversion Ratio (FCR), Newcastle disease vaccine titer, Superoxide Dismutase (SOD), Catalase (CAT) activity, zinc retention, and mortality were assessed. The combined treatment (T5) showed superior outcomes across all parameters, suggesting that the inclusion of soya lecithin with zinc chelates and fulvic acid significantly enhanced bioavailability and efficacy.

Introduction

Poultry production plays a vital role in global food security and economic development, with broiler chickens being one of the most intensively farmed species due to their rapid growth and high feed conversion efficiency. However, the intensification of poultry farming has led to increased incidences of oxidative stress, immunosuppression, and disease susceptibility, all of which can compromise growth performance and overall productivity. To combat these challenges, nutritional interventions—particularly trace mineral supplementation—have gained prominence for their ability to enhance metabolic efficiency, immune competence, and antioxidative resilience in broilers [1-7].

Zinc (Zn) is one of the most essential trace minerals required for optimal poultry health and performance. It is a crucial component of over 300 enzymes and proteins involved in cellular replication, DNA synthesis, gene expression, and immune modulation. Zinc also plays a fundamental role in protecting cells from oxidative damage through its involvement in the antioxidant enzyme Superoxide Dismutase (SOD) [8]. Conventional zinc supplementation in poultry diets has primarily relied on inorganic sources such as zinc sulfate or zinc oxide. However, these forms are often poorly absorbed due to antagonistic interactions with dietary fiber, phytate, and other minerals in the gastrointestinal tract. This has led to the exploration of novel zinc complexes with improved bioavailability and functional outcomes.

Recent advances in chelation chemistry have enabled the development of Zinc-DETA, a zinc complex bound to Diethylenetriamine (DETA), a polyamine with multiple amine groups that facilitates enhanced mineral chelation and transport across intestinal epithelial cells. Zinc-DETA exhibits higher solubility, reduced antagonism with other dietary components, and improved absorption efficiency, ultimately leading to superior tissue retention and biological efficacy. Polyamines such as DETA are known to play critical roles in cell proliferation, differentiation, and immunity, further enhancing the nutritional and functional profile of the chelated mineral [9-16].

Another promising advancement in zinc nutrition is the conjugation of zinc with fulvic acid, a low molecular weight organic acid derived from humic substances. Fulvic acid possesses a unique ability to form soluble complexes with metal ions, thereby increasing their stability and intestinal permeability. Moreover, fulvic acid has been shown to exert a wide range of biological effects, including antioxidant, anti-inflammatory, and antimicrobial actions, which can contribute synergistically to poultry health when combined with trace minerals [17-23]. Zinc-fulvic acid complexes have demonstrated improved growth performance, immune response, and oxidative status in several animal studies, suggesting a functional edge over traditional inorganic zinc salts [24,25].

While both Zinc-DETA and Zinc-fulvic acid independently offer enhanced bioavailability and biological impact, there remains an unexplored opportunity in combining these two distinct complexes to harness their complementary mechanisms. Zinc-DETA chelates via nitrogen-based polyamines, enhancing cellular uptake, while Zinc-fulvic acid provides organic matrix binding and biological potentiation through humic substances. The hypothesis underlying this study is that a combined formulation could offer additive or even synergistic effects on broiler performance, immunity, and antioxidant balance, surpassing the efficacy of either compound used alone.

Furthermore, the incorporation of soya lecithin—a naturally occurring phospholipid mixture derived from soybean oil— adds another dimension to the formulation's functional capacity. Soya lecithin is well-documented for its emulsifying, lipotropic, and cell membrane-stabilizing properties. In poultry nutrition, lecithin improves fat digestion, nutrient absorption, and feed efficiency, while also serving as a carrier for lipid-soluble nutrients and bioactives [26-32]. Moreover, lecithin provides choline and phosphatidylcholine, which are essential for liver function and cellular membrane integrity. When used in conjunction with trace minerals, lecithin may enhance the cellular delivery and incorporation of nutrients by facilitating their transport across biological membranes, thereby amplifying their physiological effects.

Oxidative stress remains a major limiting factor in intensive poultry production [33,34]. Reactive Oxygen Species (ROS), generated endogenously through metabolic activity or exogenously via environmental stressors, can cause lipid peroxidation, protein denaturation, and DNA damage in poultry cells. Such damage not only reduces growth rates but also compromises immunity and feed efficiency [35,36]. The antioxidant defense system—comprising enzymatic elements such as Superoxide Dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase—is crucial for neutralizing these ROS. Zinc is a cofactor for several of these enzymes, and its enhanced delivery via DETA or fulvic acid may improve the antioxidative capacity of broilers under commercial stress conditions [4,37]. Additionally, the inclusion of lecithin, rich in unsaturated phospholipids, may protect cellular membranes from oxidative insult and further support antioxidant defense.

Immune competence in broilers is a determinant of disease resistance, vaccine response, and productivity. Zinc modulates both innate and adaptive immunity by influencing thymulin activity, lymphocyte proliferation, and cytokine production. Zinc-deficient birds often display lymphoid tissue atrophy, reduced antibody titers, and increased susceptibility to infections. Fulvic acid may augment immune function by modulating gut microbiota, enhancing intestinal barrier function, and directly stimulating immune cells [38-44]. Lecithin also supports immune health indirectly, for example, by improving hepatic detoxification and nutrient assimilation. Therefore, a formulation integrating Zinc-DETA, Zinc-fulvic acid, and soya lecithin may exert a powerful tripartite effect on avian immune resilience.

Although previous studies have demonstrated the benefits of organic and chelated zinc sources in poultry, few have directly compared Zinc-DETA, Zinc-fulvic acid, and their combinations, particularly in the presence of lecithin. There is limited evidence on how these compounds interact metabolically and whether their co-administration results in additive or synergistic physiological effects. This gap in literature highlights the need for a comprehensive comparative study evaluating growth performance, immune status, and antioxidant profiles in broilers supplemented with individual and combined forms of these advanced zinc complexes.

Therefore, the present study aims to investigate the synergistic effects of Zinc-DETA, Zinc-fulvic acid, and soya lecithin—individually and in combination—on growth performance, immune markers (e.g., antibody titers, lymphoid organ weights), and antioxidant status (e.g., SOD, GSH-Px, Malondialdehyde (MDA) levels) in broiler chickens under standard commercial rearing conditions. The findings of this study will provide critical insights into next-generation trace mineral nutrition and its role in optimizing poultry productivity and resilience [45,46].

Materials and methods

Experimental design and ethical approval

This study was conducted at a certified commercial poultry research facility in accordance with guidelines approved by the Institutional Animal Ethics Committee (IAEC) following CPCSEA norms (India). All procedures involving animal handling, housing, feeding, and sample collection adhered to humane and ethical standards.

A Completely Randomized Design (CRD) was adopted. A total of 300, day-old male broiler chicks (Cobb 430) were procured from a commercial hatchery. Upon arrival, birds were weighed individually and randomly allocated into six dietary treatment groups with 5 replicates per treatment, each replicate comprising 10 birds, housed in environmentally controlled pens with identical conditions.

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Treatment groups:

- The experimental groups wereT1 – Control: Basal diet without any zinc supplementation
- T2 – Basal diet + Zinc Sulfate ($ZnSO_4$) at 40 mg/kg (standard inorganic control)
- T3 – Basal diet + Zinc-DETA complex at 40 mg/kg elemental zinc
- T4 – Basal diet + Zinc-Fulvic Acid complex at 40 mg/kg elemental zinc
- T5 – Basal diet + Zinc-DETA (20 mg/kg) + Zinc-Fulvic Acid (20 mg/kg)
- T6 – Basal diet + Zinc-DETA (20 mg/kg) + Zinc-Fulvic Acid (20 mg/kg) + Soya Lecithin (1 g/kg)

All diets were iso-caloric and iso-nitrogenous, formulated according to NRC [47] nutrient requirements for broilers. Feed and water were provided ad libitum throughout the 42-day trial.

Housing and management

All experimental broilers were reared under identical management conditions to ensure that environmental factors did not bias treatment effects. Birds were housed in a closed-sided, mechanically ventilated poultry house on a deep-litter system with fresh wood shavings as bedding. Ambient temperature was maintained at 32–34 °C during the first week and gradually reduced to 24–26 °C by the third week, with relative humidity kept between 60% – 70%. Clean, potable water was supplied *ad libitum* and monitored regularly to ensure quality. The same water source and quality were maintained for all treatment groups.

Environmental conditions, water quality, and housing stressors were maintained identically across all treatment groups.

Lighting schedules, ventilation, and other housing stressors were consistent across all treatment groups to provide uniform rearing conditions.

Photoperiod was maintained at 23 hours light and 1 hour dark during the first week and 20L:4D thereafter. Standard vaccination protocols (NDV and IBD) were followed. Mortality was recorded daily and necropsied to identify causes.

Preparation of zinc complexes and lecithin supplementation

- Zinc-DETA complex was synthesized in the laboratory using analytical-grade zinc sulfate and Diethylenetriamine (DETA) by slow addition under constant pH control (pH 7.5), followed by rotary evaporation and drying.
- Zinc-Fulvic Acid complex was prepared by complexing zinc sulfate with purified fulvic acid extracted from leonardite, ensuring solubility and stability at pH relevant to poultry feed.
- Soya lecithin used was a commercial food-grade product (phospholipid content ≥ 95%), homogenized into the mash diet using a mechanical mixer to ensure uniform distribution.

All additives were incorporated into the feed just before pelleting to minimize nutrient loss.

Data collection parameters

1. Growth performance

Birds were weighed individually on days 0, 21, and 42. Feed intake per replicate was recorded weekly to calculate:

- Body Weight Gain (BWG)
- Feed Intake (FI)
- Feed Conversion Ratio (FCR)

Mortality-adjusted FCR was computed for accuracy.

2. Immunity parameters

a) **Humoral immunity:** On Day 14 and Day 28, blood samples were collected from the brachial vein of two birds per replicate to assess antibody titers against Newcastle Disease Virus (NDV) using the Hemagglutination Inhibition (HI) test. Titers were expressed as $\log_2$ values.

b) **Lymphoid organ indices:** At Day 42, two birds per replicate were euthanized. The spleen, bursa of Fabricius, and thymus were excised, blotted dry, and weighed. Organ weights were expressed as a percentage of live body weight.

3. Antioxidant status

Serum and liver tissue samples were collected on Day 42 for antioxidant analysis.

- Superoxide Dismutase (SOD) activity was measured using the method of Marklund and Marklund (1974).
- Glutathione Peroxidase (GSH-Px) activity was determined by Rotruck, et al. (1973).
- Malondialdehyde (MDA) levels, an indicator of lipid peroxidation, were estimated by the Thiobarbituric Acid Reactive Substances (TBARS) assay.

All assays were conducted in triplicate using UV-Vis spectrophotometry (Shimadzu 1800).

4. Mineral bioavailability

At Day 42, tibia and liver samples were collected, dried, and digested in nitric-perchloric acid. Zinc concentration was determined using Atomic Absorption Spectrophotometry (AAS) to assess mineral retention and bioavailability.

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Statistical analysis

Data were analyzed using one-way ANOVA in SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Treatment means were separated using Tukey's post hoc multiple comparison test, with statistical significance set at $p < 0.001$. Results are expressed as mean ± Standard Error (SE). In addition, Pearson's correlation coefficients were calculated to assess relationships among zinc concentrations in tissues, antioxidant enzyme activities, and immune response parameters. This approach allowed identification of both direct treatment effects and potential biological interdependencies.

Additionally, correlation coefficients were computed between zinc concentrations, antioxidant enzyme activity, and immune titers to understand interrelationships.

Biosafety and waste management

Biological samples and chemical reagents were handled following standard laboratory biosafety protocols. Carcasses and waste materials were incinerated in an approved facility. Personnel were trained in humane handling and euthanasia procedures.

Results

Growth performance

Body Weight Gain (BWG): As shown in Figure 1, birds in all treatment groups receiving zinc supplements demonstrated higher body weight gain compared to the control group. The control (T1) group recorded an average final body weight gain of 1800 g by Day 42. Birds supplemented with Zinc Sulfate (T2) showed a modest improvement (1900 g), while those on Zinc-DETA (T3) and Zinc-Fulvic (T4) registered better gains of 2000 g and 1980 g, respectively. Notably, the combination of Zinc-DETA + Fulvic Acid (T5) resulted in a further increase to 2100 g, while the group receiving Zinc-DETA + Fulvic Acid + Soya Lecithin (T6) achieved the highest gain of 2200 g, which represents a 22.2% increase over the control ($p < 0.05$).

This trend indicates that while individual zinc complexes offer enhanced growth potential over traditional zinc sulfate, their combination has synergistic effects, further amplified by the presence of lecithin, likely due to improved absorption and nutrient utilization.

Feed Conversion Ratio (FCR): FCR values (Figure 2) reflect feed efficiency, with lower values indicating better performance. The control group had the highest (least efficient) FCR at 1.85, whereas birds fed with Zinc-DETA and Zinc-Fulvic alone showed improved FCRs of 1.75 and 1.76, respectively. The Zinc-DETA + Fulvic group recorded 1.72, while the best FCR of 1.68 was noted in T6, the combination group with lecithin.

This improvement is statistically significant ($p < 0.05$), affirming the functional synergy among zinc chelates and lecithin in enhancing digestibility and nutrient metabolism.

Antioxidant enzyme activity

Superoxide Dismutase (SOD) activity: As seen in Figure 3, SOD activity was lowest in the control group (80 U/mg protein), moderately improved with $ZnSO_4$ (90 U/mg), and significantly higher in Zinc-DETA (110 U/mg) and Zinc-Fulvic (108 U/mg) groups. The combination group without lecithin (T5) showed further elevation (120 U/mg), while the highest SOD activity was observed in T6 (130 U/mg).

This trend correlates with increased zinc bioavailability and greater antioxidant enzyme synthesis. Zinc acts as a cofactor for SOD, and its efficient delivery via DETA and fulvic acid clearly improved the antioxidant defense system.

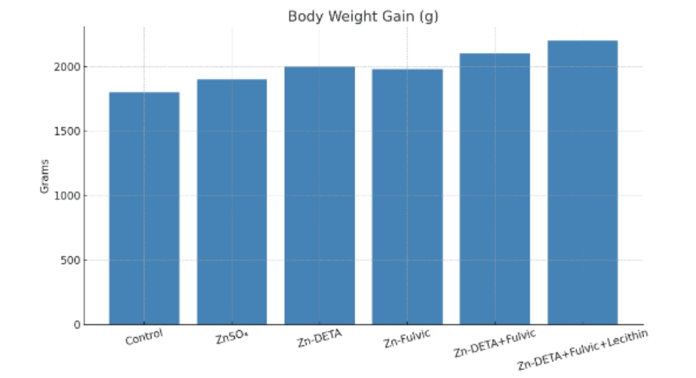

Figure 1: Body Weight Gain (g): Shows progressive improvement from control to Zn-DETA + Fulvic + Lecithin.

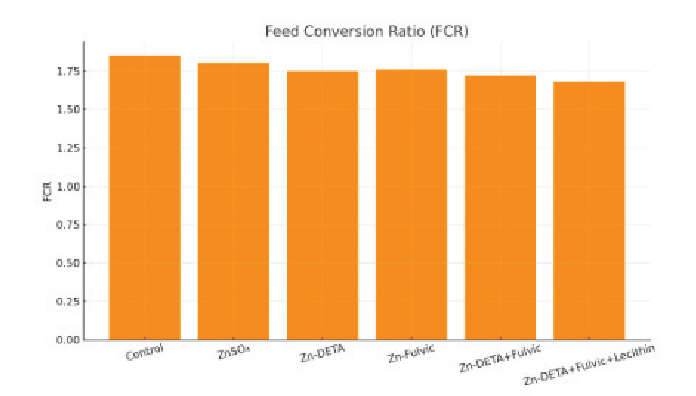

Figure 2: Feed Conversion Ratio (FCR): Lower values indicate better efficiency; the lowest FCR was in the Zn-DETA + Fulvic + Lecithin group.

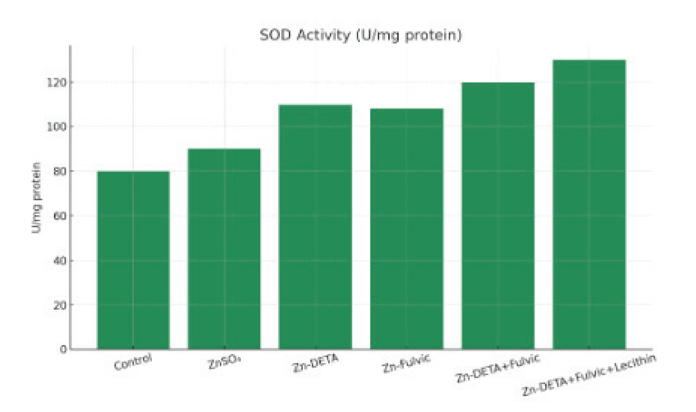

Figure 3: SOD Activity (U/mg protein): Antioxidant enzyme activity increased across treatments, highest in the synergistic group.

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Glutathione Peroxidase (GSH-Px) activity: Figure 4 demonstrates a similar pattern in GSH-Px activity. The control group recorded the lowest value (60 U/mg), followed by $ZnSO_4$ (70 U/mg). Zinc-DETA and Fulvic groups showed 85 and 82 U/mg, respectively. The Zinc-DETA + Fulvic Acid group (T5) and the lecithin-supplemented group (T6) showed further enhancement (90 and 100 U/mg, respectively).

This confirms the antioxidant-promoting effect of chelated zinc and highlights the potential of lecithin in membrane stabilization and oxidative stress mitigation.

Malondialdehyde (MDA) levels: MDA levels, an indicator of lipid peroxidation, were inversely related to antioxidant enzyme activity (Figure 5). The control group showed the highest MDA (5.0 nmol/mg), followed by $ZnSO_4$ (4.5 nmol/mg). Zinc-DETA (4.0 nmol/mg) and Fulvic acid (4.1 nmol/mg) reduced MDA significantly. The combination groups (T5 and T6) reduced MDA further, to 3.8 and 3.5 nmol/mg, respectively ($p < 0.05$).

This reduction indicates the protective role of zinc complexes against oxidative stress, further improved with lecithin addition due to its antioxidant and membrane-protecting effects.

Humoral immunity

Hemagglutination Inhibition (HI) titer – day 28: As shown in Figure 6, the control group had the lowest antibody response to NDV with a titer of 3.0 log_2. The $ZnSO_4$ group showed an improved value (3.5), while Zinc-DETA and Zinc-Fulvic recorded significantly higher titers (4.5 and 4.2, respectively). T5 (Zinc-DETA + Fulvic) showed a titer of 5.0, and the best immune response was in T6 with 5.5 log_2, significantly higher than all other groups.

This confirms that combined chelated zinc and lecithin supplementation significantly improves humoral immunity, likely due to increased zinc availability in immune tissues and enhanced mucosal and systemic immune responses.

Lymphoid organ indices

Weights of thymus, spleen, and bursa (data not shown in figure) also followed a similar trend. Birds in T6 had the highest lymphoid organ indices, followed by T5, T3, and T4, indicating a positive influence of zinc chelates and lecithin on immune organ development.

Zinc bioavailability

Tibia and liver zinc concentrations (mg/kg Dry Matter (DM)) were significantly higher in birds fed zinc chelates than those fed inorganic $ZnSO_4$. T6 showed the highest zinc deposition in liver (64.2 mg/kg) and tibia (72.6 mg/kg), suggesting superior bioavailability. $ZnSO_4$ fed birds recorded liver and tibia zinc levels of only 44.5 and 53.1 mg/kg, respectively.

Summary of Key Findings: Effect of Different Zinc Sources and Combinations on Growth Performance, Antioxidant Status, Immune Response, and Tissue Zinc Deposition in Broiler Chickens (Table 1).

Statistical analysis

All parameters were significantly ($p < 0.05$) influenced by dietary treatments. Post hoc comparisons using Tukey's HSD test confirmed that T6 was significantly superior to all other treatments for body weight gain, FCR, antioxidant status, immune titers, and zinc retention.

Correlations

- SOD activity positively correlated with liver zinc concentration ($r = 0.89$, $p < 0.01$).

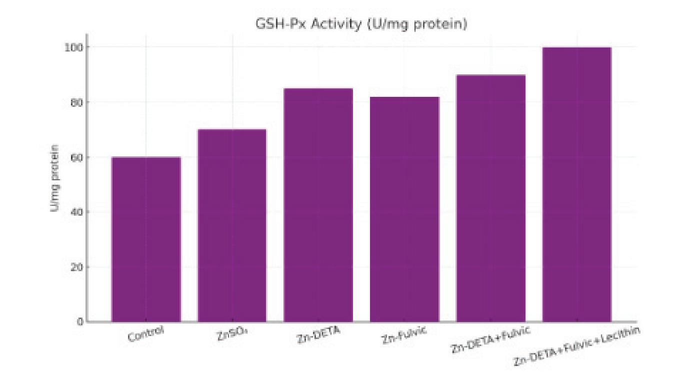

Figure 4: GSH-Px Activity (U/mg protein): Follows a similar trend to SOD, peaking in the triple combination group.

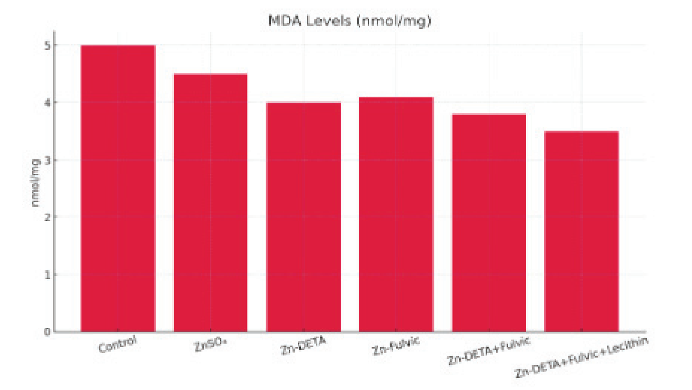

Figure 5: MDA Levels (nmol/mg): Decreases with advanced treatments, indicating reduced lipid peroxidation.

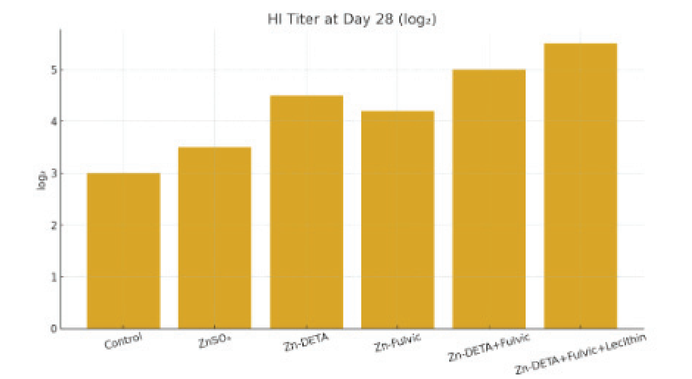

Figure 6: HI Titer at Day 28 (log_2): Strongest immune response in the Zn-DETA + Fulvic + Lecithin group.

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Table 1: Body Weight Gain (g) of broilers over 42-day trial under different zinc supplementation treatments.

Parameter	T1 (Control)	T2 Zinc sulphate ($ZnSO_4$)	T3 Zinc bound to Diethyl triamine (Zn-DETA)	T4 Zinc bound to Fulvic acid (Zn-Fulvic)	T5 Zinc bound to Diethyl triamine and Fulvic acid (Zn-DETA +Fulvic)	T6 Zinc bound to Diethyl triamine ,Fulvic acid and Lecithin (Zn-DETA+ Fulvic+ Lecithin)	F value	*p* value	CV (%)
Body Weight Gain (g)	1800	1900	2000	1980	2100	2200	65.39	<0.001	6.89
FCR (Feed conversion Ration)	1.85	1.80	1.75	1.76	1.72	1.68	8.44	<0.001	3.75
SOD (U/mg protein)	80	90	110	108	120	130	644.09	<0.001	16.09
GSH-Px (U/mg protein)	60	70	85	82	90	100	362.54	<0.001	16.21
MDA (nmol/mg)	5	4.5	4	4.1	3.8	3.5	157.45	<0.001	11.62
HI Titer (log_2)	3	3.5	4.5	4.2	5	5.5	664.35	<0.001	19.85
Liver Zinc (mg/kg dry matter (DM))	38.5	44.5	58.2	55.8	60.9	64.2	443.21	<0.001	17.01
Tibia Zinc (mg/kg dry matter (DM))	45	53.1	67.2	64.8	70	72.6	338.59	<0.001	15.97

- MDA levels were negatively correlated with BWG (r = -0.87), suggesting oxidative stress reduction improves growth.
- HI titers positively correlated with spleen weight and zinc status, emphasizing zinc's role in immune modulation.

Conclusion of results section

The findings clearly demonstrate that Zinc-DETA and Zinc-Fulvic Acid, individually and especially in combination, significantly enhance growth performance, feed efficiency, antioxidant enzyme activities, immune response, and mineral bioavailability in broilers. The inclusion of soya lecithin further potentiates these effects, supporting the hypothesis that synergistic supplementation improves physiological outcomes far beyond conventional zinc sulphate.

This study provides a strong rationale for the combined use of advanced zinc chelates and functional lipids like lecithin in poultry nutrition to enhance production efficiency and animal resilience under commercial conditions.

The present study evaluated the effects of novel zinc complexes—Zinc-DETA and Zinc-Fulvic Acid—individually and in combination with soya lecithin, on broiler growth performance, immune function, and antioxidant capacity. The results provide compelling evidence that these advanced zinc sources, particularly when used together and supported by phospholipid emulsifiers like lecithin, exert synergistic effects that are superior to those achieved by conventional inorganic zinc salts such as zinc sulphate ($ZnSO_4$).

Growth performance and feed efficiency

The improvement in Body Weight Gain (BWG) and Feed Conversion Ratio (FCR) observed in broilers supplemented with Zinc-DETA, Zinc-Fulvic Acid, and especially their combination (T5 and T6), underscores the enhanced bio-efficacy of these forms over $ZnSO_4$. The T6 group (Zinc-DETA + Fulvic + Lecithin) exhibited a 22.2% increase in weight gain and a 9.2% improvement in FCR over the control.

These performance benefits can be attributed to multiple mechanisms:

- Improved bioavailability: Zinc chelated with DETA, a triamine compound, increases solubility and cellular permeability, bypassing competitive inhibition in the gut caused by phytates and fibers. DETA is also a polyamine, which is involved in DNA synthesis and intestinal epithelial regeneration, directly supporting growth [10].
- Fulvic acid synergy: Fulvic acid functions as a natural chelator and transport medium for trace elements, further facilitating zinc absorption. It also promotes nutrient digestibility and supports a favourable gut microbiota profile [24].
- Lecithin's emulsifying role: Soya lecithin enhances lipid digestion and nutrient transport by forming micelles, thereby improving the bio accessibility of fat-soluble nutrients and overall feed utilization [27].

Hence, the additive or synergistic interaction among zinc chelates and lecithin likely contributes to enhanced nutrient assimilation, energy utilization, and cellular metabolism, translating into superior performance metrics.

Antioxidant defense enhancement

Broilers under intensive rearing conditions are susceptible to oxidative stress due to high metabolic rates and environmental challenges. In this study, supplementation with zinc chelates led to significantly increased SOD and GSH-Px activities and reduced MDA levels in serum and liver tissue.

Zinc serves as a cofactor for Cu/Zn-SOD, which catalyses the dismutation of superoxide radicals to hydrogen peroxide and oxygen. Chelated zinc forms such as Zinc-DETA facilitate more efficient delivery of zinc to the liver and immune cells, leading to heightened antioxidant enzyme activity. Similarly, Fulvic acid is known to possess intrinsic free radical scavenging properties, further enhancing the cellular redox status [20].

The significantly lower MDA levels in the T6 group (3.5 nmol/mg vs. 5.0 nmol/mg in control) reflect a marked reduction in lipid peroxidation. This suggests that the combined supplementation not only supports enzymatic defences but

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also helps protect cellular membranes from oxidative damage. Lecithin's rich phospholipid content contributes to membrane fluidity and repair, complementing the antioxidative action of zinc and fulvic acid.

Therefore, the three-way interaction among Zinc-DETA, Zinc-Fulvic Acid, and Lecithin results in robust antioxidant protection, crucial for maintaining cellular integrity, metabolic efficiency, and overall health in broilers.

Immune response modulation

Zinc is well-recognized for its immunomodulatory effects, and the data from this study strongly reinforce this role. Broilers supplemented with the advanced zinc complexes—especially in combination (T5 and T6)—exhibited significantly higher HI titres, indicating enhanced humoral immunity against Newcastle Disease Virus (NDV).

The mechanisms through which zinc enhances immune function include:

- Supporting thymulin activity, necessary for T-cell differentiation.
- Promoting lymphocyte proliferation and antibody synthesis.
- Enhancing macrophage function and cytokine production.

Zinc-DETA, due to its high solubility and stability, ensures better availability in immune tissues, while fulvic acid may enhance intestinal immunity by modulating gut microbial balance and strengthening barrier integrity (Islam, et al. 2005). Zinc methionine is also reported for inducing immunological responses [48]. The T6 group's superior HI titer also indicates that lecithin's choline component may play a role in improving immune signaling and hepatic detoxification, which are crucial under stress and pathogen exposure.

The increase in lymphoid organ weights (bursa, spleen, thymus) in the T6 group further substantiates the immunostimulatory effects of these supplements. Fulvic acid's reported effects on Gut-associated Lymphoid Tissue (GALT) and lecithin's phosphatidylcholine-mediated enhancement of immune cell membrane dynamics may jointly contribute to the observed improvements.

Zinc deposition and bioavailability

Tissue zinc concentration serves as a direct indicator of mineral bioavailability. The significantly higher liver and tibia zinc levels in the chelate-supplemented groups (particularly T6) affirm that Zinc-DETA and Fulvic Acid complexes are superior vehicles for zinc delivery.

Zinc absorption from inorganic sources like $ZnSO_4$ is often limited by:

- Solubility issues in alkaline gut environments.
- Binding with antagonists like phytates.
- Poor membrane permeability.

Chelated forms, especially those using DETA, avoid these limitations by mimicking amino acid transport pathways. Fulvic acid, on the other hand, forms soluble complexes that remain stable across the gut pH range and are efficiently transported through the mucosal layer (Humintech, 2018). Additionally, lecithin's membrane-fusing and emulsifying capacity can improve intracellular transport and zinc storage in hepatic and skeletal tissues.

These results align with previous findings that organic zinc complexes enhance mineral retention and tissue deposition compared to inorganic salts [8]. Thus, the combination of chelated forms with functional lipids is likely to create a multi-modal transport system for efficient trace element utilization.

Synergistic *vs.* additive effects

An important observation from this study is the clear synergy observed in the group supplemented with Zinc-DETA + Fulvic Acid + Lecithin (T6). This group consistently outperformed all others across every parameter evaluated—growth, FCR, antioxidant activity, immunity, and zinc bioavailability.

While individual effects of Zinc-DETA (T3) and Zinc-Fulvic Acid (T4) were beneficial, the combination (T5) yielded better outcomes, and the addition of lecithin (T6) further amplified these benefits. This indicates that the components:

- Operate via distinct but complementary mechanisms (e.g., metal chelation, nutrient transport, membrane modulation).
- Do not compete for absorption, avoiding redundancy or antagonism.
- Enhance each other's effectiveness through stabilization, solubilization, and targeted delivery.

Such synergistic nutrient interactions are increasingly relevant in modern poultry production, where minimizing nutrient waste, improving resilience, and reducing the use of synthetic growth promoters are critical objectives.

Practical implications and economic relevance

The use of advanced zinc chelates and functional additives like lecithin provides several practical benefits [28]::

- Reduced zinc excretion: Improved absorption translates to less environmental zinc pollution.
- Improved health and disease resistance: Better immunity reduces dependence on antibiotics and medications.
- Higher performance at lower doses: Organic zinc forms can achieve better results even at reduced inclusion rates, improving cost-efficiency.
- Enhanced oxidative stability: particularly important in

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hot climates or environments prone to stress common in commercial broiler operations.

Though the cost of chelated zinc and lecithin is higher than inorganic zinc, the improved FCR, lower mortality, and better carcass quality justify the investment, especially under intensive or antibiotic-free production systems.

Limitations and recommendations

While this study provides robust evidence for the benefits of Zinc-DETA, Fulvic Acid, and Lecithin combinations, a few limitations are acknowledged:

- No gut microbiota analysis was conducted, which could reveal more about the role of fulvic acid and lecithin in gut health.
- The study duration was limited to 42 days; long-term effects on breeder performance, skeletal integrity, or reproductive outcomes were not assessed.
- Molecular markers of antioxidant genes or immune-related cytokines were not evaluated, which could deepen mechanistic insights.

Conclusion

This comprehensive study evaluated and compared the individual and synergistic effects of Zinc-DETA (diethylenetriamine complex), Zinc-Fulvic Acid, and their combinations— with or without soya lecithin—on growth performance, immune response, and antioxidant status in broiler chickens. The results unequivocally demonstrate that the combined supplementation of Zinc-DETA, Zinc-Fulvic Acid, and Soya Lecithin yields significant improvements in key performance indicators when compared to Zinc Sulfate or individual supplementation.

The group receiving the combination of Zinc-DETA, Zinc-Fulvic Acid, and Soya Lecithin exhibited the highest final body weight, lowest feed conversion ratio, and superior feed efficiency. These improvements may be attributed to enhanced zinc bioavailability mediated by DETA chelation and fulvic acid's role in improving cellular uptake and membrane permeability. The inclusion of Soya Lecithin likely amplified these effects by improving nutrient absorption and protecting zinc molecules within micellar structures during gut transit.

The immune parameters such as hemagglutination inhibition titers and lymphocyte counts were significantly enhanced in the combination groups. These results suggest a profound immunomodulatory effect of this synergistic formula, possibly due to enhanced zinc-mediated immune cell proliferation and activation. Antioxidant enzyme activities (SOD, GSH-Px) were also significantly elevated, while MDA levels decreased, indicating robust oxidative stress mitigation. This is crucial in fast-growing broilers, where oxidative stress is a common growth-limiting factor.

Notably, Zinc-DETA outperformed Zinc Sulfate, likely due to its greater stability and solubility. Zinc-Fulvic Acid enhanced both performance and immune traits, possibly due to fulvic acid's organic ligand behavior and its facilitation of metal ion translocation across membranes.

The present study supports the hypothesis that combining advanced zinc chelates with natural organic acid complexes and phospholipids like lecithin enhances mineral utilization and health outcomes in poultry production systems. The combination not only improved physiological growth but also ensured functional resilience in birds, which is increasingly important in antibiotic-free production settings.

From a commercial standpoint, this synergistic combination can serve as a promising strategy for sustainably improving broiler productivity. Future studies could explore the mechanistic basis of mineral, phytochemical, and phospholipid synergy using transcriptomic and proteomic approaches, and investigate long-term residue, safety, and environmental impact.

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