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EFFECT OF DIETARY INCLUSION OF PURE *ALPHA MONO LAURIC ACID* ON ANTI-OXIDANT STATUS IN COMMERCIAL BROILER CHICKEN UNDER HOT CLIMATIC CONDITION

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ABSTRACT

An experiment was structured to evaluate the effects of dietary inclusion of pure alpha mono-lauric acid (α -MLA) as an alternative to antibiotic growth promoters (AGP) on antioxidant status in commercial broiler chickens reared in hot climate conditions. A total of 250-day-old broiler chicks were randomly allocated across five dietary treatment groups, with each group containing 10 replicates of five chicks each. The birds were housed in battery brooders under consistent management practices and fed their assigned diets from day one through 35 days of age. The basal diet (BD) provided during the pre-starter (1-14 days), starter (15-28 days), and finisher (29-35 days) phases consisted of a standard corn-soybean meal with antibiotic growth promoter. The other four diets were formulated by adding alpha mono lauric acid at incremental levels of 0.25g/kg, 0.5g/kg, 0.75g/kg, and 1g/kg of feed. Blood samples were taken from one bird per replicate on day 35 to assess cortisol levels and antioxidant enzymes levels. During the experimental phase (0-35 days), the lowest and highest recorded temperatures were 26.00°C and 42.85°C, respectively, with relative humidity ranging from 18.14% to 73.00%. Significant differences ($P < 0.05$) were observed in the activity of certain antioxidant enzymes, such as glutathione peroxidase (GPx), superoxide dismutase (SOD), and lipid peroxidation (MDA), among the treatment groups. The activity of glutathione reductase (GRx) did not differ significantly ($P > 0.05$) across the groups. Dietary supplementation with α -MLA significantly ($P < 0.05$) affected cortisol levels. Based on the results it was concluded that supplementation of alpha mono-lauric acid at 0.25g/kg to 0.5g/kg resulted in higher antioxidant enzyme responses, and lower cortisol hormone level. Therefore, pure alpha mono-lauric acid at 0.25g/kg to 0.5g/kg in the diet of broilers can increase the antioxidant status of broiler birds.

Key words: Alpha mono-lauric acid, Antibiotic growth promoter, Broilers, Hot climatic condition, Anti-oxidant Status and Cortisol.

Introduction

Commercial chicken farming exposes broilers to various nutritional, environmental, and biological stressors, compromising biosecurity and affecting birds health. Traditionally, antibiotics have been used to manage bacterial and viral infections, but their prolonged use poses

risks, such as weakening natural immunity, fostering antibiotic resistance, and polluting the environment. Antibiotics also face limitations due to their species-specific actions, which may not target all pathogens effectively.

Short- and medium-chain fatty acids (MCFAs),

including alpha-mono lauric acid (α -MLA), offer a promising alternative, boosting immunity and promoting growth (De Gussem *et al.*, 2021; Mustafa, 2018). α -MLA, a monoglyceride found in coconut oil and palm kernel oil, is stable under high heat and acidity in the digestive system, making it suitable for poultry diets. Recognized as a safe food additive by the United States FDA, α -MLA (synonyms: monolaurin, glycerol monolaurate, glyceryl laurate) is known for its strong antibacterial, antiviral, antioxidant, and immunostimulant properties (Jiang *et al.*, 2018; Welch *et al.*, 2020; Jackman *et al.*, 2020). Its mechanism involves disrupting the cell membranes of pathogens by solubilizing lipids. Hence a study was conducted to evaluate the effect of dietary α -MLA on the antioxidant status of broilers under hot climatic conditions.

Materials and Methods

Experimental Design

To conduct the above study, 250-day-old straight run broiler chicks (cobb 400) were randomly distributed into five dietary treatment groups each having 10 replicates with 5 chicks each. The chicks were housed in battery brooder cell (2'×2'). Birds were immunized against Newcastle disease (ND) with Lasota vaccine on 7th (primary) and 28th (booster) days of age, and infectious bursal disease (intermediate – Georgia strain) vaccine on 14th (primary) and 21st (booster) days of age.

Diet Formulation

Five experimental diets were formulated: The basal diet (BD) was prepared with the standard corn-soybean meal-based diet including an antibiotic growth promoter (AGP: Bacitracin Methylene Disalicylate), which served as the control diet (Table 1). The remaining four experimental diets were prepared without AGP by supplementing the standard corn-soybean meal-based diet with pure alpha-mono-lauric acid (Fine X 3060, Fine Organics Industries Mumbai) at rates of 0.025%, 0.05%, 0.075%, and 0.1% respectively. Feed was offered *ad libitum* to the birds during pre-starter (0-14 d), starter (15-28 d) and finisher (29-35 d) period to meet the nutrient requirements as per BIS-2007.

Collection of blood

Blood was collected from one bird per replicate on 35th day of age. Blood samples were collected aseptically from brachial vein of birds with the help of sterilized needles and blood was collected in clean sterilized glass tubes and kept in slanted position at room temperature for serum collection. The collected serum samples were then centrifuged at 3000 rpm for 5 minutes and transferred

Table 1: Ingredient composition (%) and calculated nutrient composition of the standard basal diets for broilers (100 kg).

Ingredient	PS	S	F
Maize (%)	53.05	53.74	58.38
Oil-veg (%)	2.74	4.30	5.00
Deoiled soyabean meal (%)	40.24	38.00	32.55
Salt (%)	0.35	0.30	0.30
Dicalcium phosphate (%)	1.80	1.90	2.00
Lime stone powder (%)	1.20	1.20	1.20
DL-Methionine (%)	0.15	0.15	0.13
L-lysine HCL (%)	0.06	0.00	0.03
Growth promoter ¹ (%)	0.05	0.05	0.05
Choline chloride, 75%	0.06	0.06	0.06
Toxin binder ² (%)	0.05	0.05	0.05
Trace mineral mixture ³ (%)	0.15	0.15	0.15
Vitamin premix ⁴ (%)	0.05	0.05	0.05
Coccidiostat ⁵ (%)	0.05	0.05	0.05
Total	100	100	100
Nutrient composition (calculated values)			
ME, (Kcal/kg)	2992.91	3097.12	3183.80
Protein (%)	23.01	22.03	20.00
Lysine, (%)	1.31	1.20	1.10
Methionine (%)	0.50	0.49	0.45
Calcium, (%)	1.00	1.09	1.02
Available phosphorous (%)	0.46	0.45	0.46
PS: Pre-Starter (1-14 days); S: Starter (15-28 days); F: Finisher (29-35 days) 1. Five gram of Growth promoter provided per kg diet: Bacitracin methylene Di salicylate (BMD), 2. Five gram of toxin binder provided per kg diet: Acetic acid 2mg, Formic acid 2mg, propionic acid 2mg, citric acid 2mg, Benzoic acid 1mg, Sorbic acid 0.25 mg, HSCAS 9.1mg., 3. Each gram of trace mineral mixture provided per kg diet: Manganese 1.1 mg, Zinc 1mg, Iron 110mg, Copper 20mg, Iodine 2.5mg, Cobalt 1mg, Chromium 0.4mg and Selenium 1.5mg, 4. Vitamin pre-mix provided per kg diet: Riboflavin 25mg, Vitamin B1 1mg, Vitamin B6 2mg, Vitamin B12 40mg, Niacin 15mg, Vitamin A 20000 IU, Vitamin D3 3000 IU, Vitamin K 2mg and 5. Five gram of coccidiostat provided per kg diet: Maduramicin 5 mg			

to 2 ml Eppendorf tubes which were stored at -20°C until sample analysis for estimation of serum cortisol and antioxidant enzymes.

Antioxidant Enzyme Estimation

The activities of key antioxidant enzymes, including Glutathione Peroxidase (GPx), Glutathione Reductase (GRx), Superoxide Dismutase (SOD), and lipid peroxidation (LP), were assessed using standardized methods. GPx activity was measured based on Paglia and Valentine (1967), involving the reaction of serum with

Table 2: Effect of dietary supplementation of Pure alpha mono-lauric acid on Antioxidant status in broilers during summer.

DIET	SOD	GP	GR	LP
STANDARD DIET	2.55 ^b	207.900 ^b	226.40	4.40 ^a
α -MLA-0.25g/kg	3.70 ^a	226.90 ^a	242.10	3.77 ^b
α -MLA-0.5g/kg	3.74 ^a	223.00 ^a	242.10	3.57 ^b
α -MLA-0.75g/kg	3.51 ^a	220.90 ^a	241.00	3.67 ^b
α -MLA-1g/kg	3.54 ^a	220.60 ^a	239.70	3.63 ^b
N	10	10	10	10
SEM	0.13	1.84	2.49	0.97
P-value	0.02	0.01	0.22	0.04

SOD: Super oxidizedismutase (units/mg protein); **GP:** Glutathione peroxide (units/ml); **GR:** Glutathione reductase (units/ml); **LP:** Lipid peroxidation (nmol MDA/mg protein)
P- value: probability value. N: number of replicates (5 birds in each replicate); SEM: Standard Error Mean
SD: Control diet +0.05% antibiotic; α MLA: Control diet+0.025%; α MLA: Control diet+0.5%; α MLA: Control diet+0.75%; α MLA: control diet + 1%
Means with different superscript in a column differ significantly: (*P < 0.05)

PBS, H₂O₂, reduced glutathione, and NADPH, with absorbance changes recorded at 340 nm using an ELISA reader. GRx activity followed the Carlberg and Mannervik (1985) protocol, using oxidized glutathione, FAD, EDTA, and NADPH, with similar spectrophotometric readings. SOD activity was estimated using pyrogallol and MTT in a reaction that produced formazan crystals dissolved in DMSO, measured at 570 nm (Madesh and Balasubramanian, 1998). Lipid peroxidation was determined by quantifying malondialdehyde (MDA) using the thiobarbituric acid (TBA) assay (Ohkawa *et al.*, 1979). The resultant pink chromogen was measured at 542 nm after extraction into a butanol-pyridine mixture, with interference minimized by using an alkaline solution. These enzyme activities were expressed as units per milligram of protein or nanomoles of MDA per milligram of protein, calculated using specific extinction coefficients and formulas.

Estimation of serum cortisol:

The cortisol (COR) hormone in serum was estimated using a commercial ELISA kit (Cat. No. EA0019cC, Bioassay Technology Laboratory). The kit utilized pre-coated Chicken COR antibodies to bind cortisol in the samples. Biotinylated COR antibodies were then added, followed by streptavidin-HRP, which binds to the biotinylated antibody. After incubation, unbound components were washed off, and a substrate solution was added, producing a colour proportional to the cortisol concentration. The reaction was stopped with an acidic

Table 3: Effect of dietary supplementation of Pure Alpha mono-lauric acid on Cortisol level in broilers during summer.

DIET	CORTISOL LEVEL (ng/L)
STANDARD DIET	1.04 ^a
α -MLA-0.25g/kg	0.80 ^b
α -MLA-0.5g/kg	0.81 ^b
α -MLA-0.75g/kg	0.83 ^b
α -MLA-1g/kg	0.84 ^b
N	10
SEM	0.21
P-value	0.001

Means with different superscript in a column differ significantly: (*P < 0.05)
P- value: probability value. N: number of replicates (5 birds in each replicate); SEM: Standard Error Mean
SD: Control diet +0.05% antibiotic; α MLA: Control diet+0.025%; α MLA: Control diet+0.5%; α MLA: Control diet+0.75%; α MLA: control diet + 1%.

solution, and absorbance was measured at 450 nm using a microplate reader (Thermo Fisher Scientific, Multiscan GO).

Standards were prepared by serial dilutions of a 48 ng/mL stock solution to generate concentrations of 24, 12, 6, 3, and 1.5 ng/mL. For the assay, 50 μ L of standards and samples were added to respective wells, followed by streptavidin-HRP, and incubated at 37°C for 60 minutes. After washing, substrate solutions A and B were added, incubated for 10 minutes in the dark, and the reaction was terminated with a stop solution.

Statistical Analysis

The data were analyzed for mean, standard error, and analysis of variance (ANOVA) following the method outlined by Snedecor and Cochran (1989). Mean comparisons were performed using Duncan's Multiple Range Test (Duncan, 1955) with the Statistical Package for Social Sciences (SPSS) software, version 15.0. Statistical significance was set at P<0.05.

Data obtained were analyzed for mean, standard errors and analysis of variance as per method of Snedecor and Cochran (1989). Comparison of means were done using Duncan's multiple range test (Duncan, 1955) using software of Statistical Package for Social Sciences (SPSS) 15.0 version and significance was considered at P<0.05.

Results

From the results of experiment, there is a significant difference (P<0.05) on various antioxidant parameters such as super oxide dismutase, glutathione peroxide and lipid per oxidant but there were no influences of alpha

mono-lauric acid on glutathione reductase which can be seen in Table 2. The antioxidant activity of food additives plays a crucial role in poultry production, so the reduction in MDA by alpha-monolaurin may be owing to augmentation effects of monolaurin on gut activity, pro-inflammatory cytokines, and antimicrobial and antiviral effects (Sahel *et al.*, 2021). The present observations are in correlation with studies of Rizal *et al.*, (2017), Sahel *et al.*, (2021). Rizal *et al.*, (2017) studied the effects of dietary alpha-monolaurin on liver antioxidant enzyme activities in broilers. They found that low doses of alpha-monolaurin (0.1-0.2%) improved SOD and GPx activities in the liver, indicating enhanced antioxidant defenses. Sahel *et al.*, (2021) investigated the effects of alpha-monolaurin supplementation on serum antioxidants and concluded that MDA levels were significantly ($P<0.05$) reduced in birds given 0.25 to 1 g of alpha-mono lauric acid per kg of feed compared to the control feed.

Cortisol is released in response to stress, whether caused by environmental factors (e.g., heat, overcrowding, poor ventilation), handling, transportation, or changes in management practices. High serum cortisol values indicate that the birds are experiencing significant stress. By measuring cortisol levels, producers can monitor and mitigate stressors to improve broiler welfare and productivity. The effect of a-mono lauric acid supplementation on serum cortisol levels is shown in Table 3. Dietary supplementation of Alpha mono-lauric acid (0, 0.25, 0.5, and 1 g/kg feed) to broilers significantly ($P<0.05$) influenced the cortisol level compared with control group of birds fed with standard diet. The Cortisol level in birds fed alpha mono- lauric acid is statistically lower compared to birds fed with AGP. Similarly, Shakeri *et al.*, (2015) reported that supplementation of 3% coconut milk significantly ($P<0.05$) reduced cortisol level in the treatment group of birds when compared to control birds.

Discussions

Serum Antioxidant Enzyme Responses

Antioxidant enzymes like superoxide dismutase (SOD), catalase, glutathione reductase (GRx), and glutathione peroxidase (GPx) help protect cells from oxidative damage caused by reactive oxygen species (ROS) (Ighodaro & Akinloye, 2018). Measuring these enzymes provides insight into the oxidative stress levels in birds, which can be heightened due to environmental stressors (e.g., heat stress), feed quality, and other factors. From the results of experiment, there is a significant difference ($P<0.05$) on various antioxidant parameters such as super oxide dismutase, glutathione peroxide and lipid peroxidant but there was no influence

of alpha mono-lauric acid on glutathione reductase. The antioxidant activity of food additives plays a crucial role in poultry production, so the reduction in MDA by alpha-monolaurin may be owing to augmentation effects of monolaurin on gut activity, pro-inflammatory cytokines, and antimicrobial and antiviral effects (Sahel *et al.*, 2021). The present observations are in correlation with studies of Rizal *et al.*, (2017), Sahel *et al.*, (2021). Rizal *et al.*, (2017) studied the effects of dietary alpha-monolaurin on liver antioxidant enzyme activities in broilers. They found that low doses of alpha- monolaurin (0.1-0.2%) improved SOD and GPx activities in the liver, indicating enhanced antioxidant defences. Sahel *et al.*, (2021) investigated the effects of alpha- monolaurin supplementation on serum antioxidants and concluded that MDA levels were significantly ($P<0.05$) reduced in birds given 0.25 to 1 g of alpha-mono lauric acid per kg of feed compared to the control feed.

Cortisol level

Cortisol is released in response to stress, whether caused by environmental factors (e.g., heat, overcrowding, poor ventilation), handling, transportation, or changes in management practices (Scanes & Vizzier-Thaxton, 2017). High serum cortisol values indicate that the birds are experiencing significant stress (Nelson *et al.*, 2018). By measuring cortisol levels, producers can monitor and mitigate stressors to improve broiler welfare and productivity (Idowu *et al.*, 2021). Dietary supplementation of Alpha mono-lauric acid (0, 0.25, 0.5, and 1 g/kg feed) to broilers significantly ($P<0.05$) influenced the cortisol level compared with control group of birds fed with standard diet. The cortisol level in birds fed alpha mono- lauric acid is statistically lower compared to birds fed with AGP. Similarly, Shakeri *et al.*, (2015) reported that supplementation of 3% coconut milk significantly ($P<0.05$) reduced cortisol level in the treatment group of birds when compared to control birds.

Conclusion

The activities of antioxidant enzymes, such as glutathione peroxidase (GPx), superoxide dismutase (SOD), and lipid peroxidation (MDA), were significantly higher ($P<0.05$) in the alpha mono-lauric acid supplement group compared to the control group. Dietary supplementation with alpha mono-lauric acid significantly ($P<0.05$) affected cortisol levels.

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