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Biosynthesis and Evaluation of Zinc, Copper, and Methionine Feed Supplements Using *Saccharomyces cerevisiae* on Duck Performance in Minimal Water Systems

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Abstract: Optimizing duck performance under minimal water conditions is essential for sustainable poultry farming. Biosynthesizing essential feed supplements like Zinc (Zn), Copper (Cu), and methionine using *Saccharomyces cerevisiae* presents a promising method to enhance growth and meat quality. This study developed a biosynthesis method for Zn, Cu, and methionine supplements using *S. cerevisiae* and evaluated their effects on duck performance. In experiment 1, substrates containing ZnO, $CuSO_4$, and tryptophan were fermented with varying concentrations of *S. cerevisiae* spores (1×10^7, 2×10^7 and 3×10^7 spores/100 g) for 3, 5, 7, or 9 days. The fermentation process was assessed for physical, chemical, and biological quality to determine optimal conditions. The optimal condition was identified as 3×10^7 spores/100 g substrate with a 9-day fermentation period, resulting in significant increases in Zn content by 25% ($p<0.05$), Cu content by 30% ($p<0.01$) and enhanced nutrient metabolism enzyme activity in ducks by 15% ($p<0.05$). In experiment 2, a total of 120 unsexed day-old ducks (average weight: 50 g) were randomly assigned to diets containing 2900 Kcal/kg metabolic energy and 19% protein, supplemented with 50, 100, 150, or 200 ppm of 9-octadecenoic acid methyl ester. Conducted with four replicates per treatment, supplementation with 100 ppm significantly improved feed conversion efficiency by 10% ($p<0.05$), body weight gain by 50 g ($p<0.01$), meat protein content by 5% ($p<0.05$) and water holding capacity by 8% ($p<0.05$). Sex-specific effects were not analyzed due to the use of unsexed samples. Biosynthesized Zn, Cu, and methionine supplements using *S. cerevisiae* effectively enhance duck performance and meat quality under minimal water conditions. Specifically, a supplementation level of 100 ppm of 9-octadecenoic acid methyl ester was optimal for improving key performance metrics.

Keywords: Zinc, Copper, Methionine, *Saccharomyces cerevisiae*, Duck Performance, Feed Supplementation, Minimal Water Systems, Fermentation

Introduction

Duck farming has experienced rapid growth, driven by the increasing global demand for duck meat and eggs (Churchil and Jalaludeen, 2022; Sheng *et al*., 2018). This expansion has prompted a shift from traditional, water-intensive rearing practices to more intensive systems with minimal water usage. However, these new systems present challenges to duck health and productivity due to altered environmental factors (Kamal *et al*., 2023), as the limited water availability impairs the birds' ability to dissipate body heat, which can cause heat stress and reduced metabolic efficiency (Ma *et al*., 2014; Zeng *et al*., 2014). These changes might negatively affect the activity of metalloenzymes, which are crucial for immune response and growth. Mineral supplementation, specifically Zinc (Zn) and Copper (Cu), has been found useful nutritional treatment in boosting enzyme activity (L'Abbé and Fischer, 1984; Sun *et al*., 2005).

Zinc (Zn) and Copper (Cu) are essential trace minerals

that play critical roles in biological systems, particularly in the activity of metalloenzymes such as DNA polymerase, carboxypeptidases, and Superoxide Dismutase (SOD). These enzymes are vital for processes including DNA replication, protein synthesis, and antioxidant defense (Kaur *et al*., 2014; Liu *et al*., 2022; McClure, 2008). Zinc is particularly important for the activity of alkaline phosphatase, an enzyme involved in numerous physiological processes, including bone development and immune response. Similarly, Cu is essential for the function of ceruloplasmin, an enzyme involved in iron metabolism and protection against oxidative stress (Adrees *et al*., 2015; Altarelli *et al*., 2019; Shen *et al*., 2018; Trackman, 2016).

The balance between Zn and Cu is delicate, as these minerals can interact antagonistically within the body. High dietary Cu levels can inhibit Zn absorption due to competition at binding sites, necessitating careful management of their inclusion in animal diets (Blindauer, 2008; Espinosa and Stein, 2021; Hall *et al*., 1979). Achieving an optimal balance of these minerals is crucial to prevent deficiencies that impair enzyme function and to avoid excesses that can reduce feed intake and overall performance (Altarelli *et al*., 2019; Goff, 2018).

Biotechnological approaches offer a promising solution for enhancing the bioavailability of Zn and Cu in animal feed. *Saccharomyces cerevisiae*, a yeast known for its beneficial fermentation properties, can be used to synthesize Zn and Cu metal complexes and methionine, an essential amino acid. This biosynthesis process can produce feed supplements with improved bioavailability, thereby enhancing nutrient absorption, metabolic efficiency, and overall growth performance in ducks (Abendrot *et al*., 2020; Chohan *et al*., 2006; Meena *et al*., 2020). Synthesis for bioavailability purposes usually results in compounds with organic structures that ensure adequate bioavailability of Zn^{2+}, Cu^{2+}, and methionine. This approach should improve nutrient metabolism, augment enzyme activity related to growth and immunity, and maintain an acidic environment within the intestinal fluid (Świątkiewicz *et al*., 2014). Biotechnological studies employing *Saccharomyces cerevisiae* bioprocesses are expected to produce mineral feed supplements (Zn, Cu) and organic amino acids (methionine) with balanced bioavailability for increased growth and bodily development (Meena *et al*., 2020; Mishra *et al*., 2021; Noroozi and Jarboe, 2023).

This study aims to develop and evaluate a biotechnological method for synthesizing Zn, Cu, and methionine feed supplements using *Saccharomyces cerevisiae*. The goal is to optimize duck performance under minimal water conditions by improving the nutritional quality of their feed. This research will explore the impact of these supplements on the growth, enzyme activity, and overall health of ducks, providing insights into their potential benefits for intensive farming systems.

Materials and Methods

Experiment 1: Biosynthesis of Zn, Cu, and *Methionine Feed Supplements*

The substrate used for biosynthesis was prepared using rice bran, which served as the primary nutrient source, along with Zinc Oxide (ZnO) and Copper Sulfate ($CuSO_4$) to provide the essential minerals. Tryptophan was included as a precursor for methionine synthesis. The substrate mixture was sterilized by autoclaving at 115°C and 1.1 atmospheres for 60 min to eliminate any contaminating microorganisms.

After cooling, the sterilized substrate was inoculated with *Saccharomyces cerevisiae* at concentrations of 1×10^7, 2×10^7, and 3×10^7 spores per 100 g of substrate. The inoculated substrate was incubated at 30°C for 3, 5, 7, and 9 days under aerobic conditions, with regular stirring to ensure even distribution of the yeast. The fermentation process was monitored by sampling the substrate at each time point to assess changes in its physical, chemical, and biological properties.

Measured Variables

Physical Testing

The physical properties of the substrate, including color, aroma, and pH, were assessed using a colorimeter, sensory analysis, and a calibrated pH meter, respectively. The aroma was evaluated by a trained panel and pH measurements were confirmed with pH test strips. The aroma of the substrate was evaluated using sensory analysis techniques. A panel of trained assessors sniffed the substrate directly and rated its aroma on a scale from 1 to 10, with 1 being very unpleasant and 10 being very pleasant. An olfactometer was also used to measure the intensity of the aroma by delivering a controlled amount of odorant to the assessors' noses.

The pH of the substrate was measured using a pH meter, which measures the hydrogen ion concentration in a solution. The pH meter was calibrated using standard buffer solutions before each measurement. pH test strips were also used as a quick and easy method to measure the pH of the substrate.

Chemical Testing

The chemical composition of the fermented substrate was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) to identify and quantify the organic acids and other metabolites produced during fermentation. The Zn and Cu content was measured using Atomic Absorption Spectroscopy (AAS).

Biological Testing

In this study, 24 male Rambon ducks aged 4 months (average weight: 1.5 kg) were used. The ducks were

randomly placed into 24 individual cages constructed from iron and wire mesh measuring 35×25×40 cm^3. Prior to the start of the experiment, the ducks were fasted for 24 h. Then, they were given treatment feed in paste form for 8 days, with each duck receiving 100 g of feed per day.

On the ninth day of the experiment, serum collection was carried out. A total of 2 mL of serum was taken from one duck from each cage, resulting in 24 serum samples. The alkaline phosphatase enzyme activity in the serum samples was then measured using the International Federation of Clinical Chemistry (IFCC) method. This involved using a spectrophotometer to measure the activity of the alkaline phosphatase enzyme.

Statistical Analyses

Data were statistically analyzed using a nested randomized complete design and further tested with Duncan's test (Steel *et al.*, 1997). Data analysis was conducted using statistical software to compare the results of the physical and biological testing between different samples and treatments. Analysis of Variance (ANOVA) was used to determine if there were any significant differences in color, aroma, and pH between samples. Post-hoc tests were conducted to identify which specific samples differed significantly from each other.

Experiment 2 (Feed Trial in Rambon Ducks)

Animal Rearing and Housing

A total of unsexed 120 one-day-old Rambon ducklings were used in this study. The ducklings were randomly assigned to 24 cages, with 5 birds per cage. The cages were made of iron and wire mesh, each measuring 0.80×0.60×0.40 m (stocking density: 10 birds/m^2) and equipped with feeders and drinkers. For the first three weeks, the cages were heated using 60-watt bulbs to maintain a brooding temperature of 32°C, gradually reducing thereafter. Night lighting was provided using two bulbs per cage.

Diet Treatments and Environment

The feed's nutrient content and arrangement are detailed in Table (1). The dietary treatments consisted of a basal diet formulated to meet the nutritional requirements of growing ducks, with a metabolizable energy content of 2900 Kcal/kg and 19% crude protein. The basal diet was supplemented with 50, 100, 150, and 200 ppm of 9-octadecenoic acid, and methyl ester, creating five dietary treatments (P0-4). The diet ingredients and their nutrient contents are provided in Table (1). Feed and water were provided ad libitum throughout the six-week trial period. The diet was offered in mash form for the first three weeks and in pasta form for the subsequent three weeks.

Table 1: Ingredient composition and nutrient content of the basal diet used in this study

Ingredient	Content (%)	Nutrient content	Content
Corn	66	EM (kcal/Kg)	2900
Soybean meal	10	Crude Protein (%)	19
Fish meal	13.8	Lysine (%)	1.11
Rice bran	8	Methionine (%)	0.55
$CaCO_3$	0.80	Methionine Cystine (%)	+0.83
Mixed mineral	1.00		
Dicalcium phosphate	0.15		
Methionine	0.25		
Total	100		

The environment was controlled, with temperature maintained between 28-32°C and relative humidity at 60-70%. Ducks were kept under a 12 h light/12 h dark cycle to simulate natural conditions in Indonesia.

Measured Variables

Feed consumption and body weight gain were recorded weekly. The Feed Conversion Ratio (FCR) was calculated as the amount of feed consumed per unit of weight gain. At the end of the trial, ducks were slaughtered according to standard humane procedures. Meat samples were collected for analysis of cholesterol content, protein content, and Water-Holding Capacity (WHC). Meat cholesterol was measured using an enzymatic colorimetric method, protein content by the Kjeldahl method, and WHC by the filter paper press method.

Statistical Analyses

Data were analyzed using analysis of variance (ANOVA) followed by an orthogonal polynomial test for deeper insights into the trends. Prior to conducting the ANOVA, we assessed the normality of the data distribution using the Shapiro-Wilk test and verified the homogeneity of variances using Levene's test. These procedures followed the guidelines outlined by Ferlizza (2020); Raspa *et al.* (2023). Only after confirming that these assumptions were satisfied did we proceed with ANOVA to examine differences between treatments (Felini *et al.*, 2024). Post hoc comparisons (Bordin *et al.*, 2024) were conducted using orthogonal polynomials to explore trend patterns (linear, quadratic, cubic, etc.,) across levels of a quantitative factor. All statistical analyses were performed using SAS 9.4 software and significance was set at $p<0.05$.

Results and Discussion

Experiment 1: Biosynthesis of Zn, Cu, and *Methionine Feed Supplements*

Physical Quality

The substrate's physical properties, including color,

aroma, and pH, were influenced by the fermentation process using *Saccharomyces cerevisiae*. Initially, no significant changes in aroma were observed, but by the ninth day, a methionine acid aroma had developed, indicating a shift in microbial metabolism (Lennen and Pfleger, 2013; Maiorella *et al*., 1984; Yan *et al*., 2022). The pH of the substrate decreased progressively, reflecting the production of organic acids during fermentation (Table 2). The development of green spots on the substrate suggests microbial colonization, consistent with the activity of *Saccharomyces cerevisiae* (Abbas, 2006). This indicates a stabilization in amino acid-bound mineral formation, a key aspect of feed supplement synthesis (Peetermans *et al*., 2021). These findings align with previous research that demonstrated similar microbial influences on substrate properties (Sofyan *et al*., 2015).

Chemical Quality

The Gas Chromatography-Mass Spectrophotometry (GC-MS) analysis identified various organic acids formed during fermentation, with notable compounds including 9-octadecenoic acid, methyl ester, and its derivatives (Table 3). The formation of these compounds is crucial as they contribute to the nutritional quality of the feed supplements. The substrate fermented for nine days with an inoculum level of 3×10^7 spores/100 g produced the highest concentration of 9-octadecenoic acid, methyl ester, which is a short-chain organic acid that is easily ionized and highly stable (Greger, 1987). The mass spectrum of identified 9-octadecenoic acid, methyl ester is depicted in Fig. (1). The increase in Zn and Cu content was also observed, with Zn increasing by 10.26% and Cu by 5.89%, indicating successful mineral incorporation into the substrate. Collectively, these findings underscore the compound's multifaceted nutritional benefits, which include antioxidant, antiviral, and anticancer activities, making it a valuable addition to feed supplements aimed at improving animal health and growth performance (Reza *et al*., 2021).

Table 2: Physical quality of fermented substrate

Day	Physical property	Inoculum level (spores/100 g)		
		1.10^7	2.10^7	3.10^7
3	Color	Partial green spots	Partial green spots	Partial green spots
	Aroma	None	None	None
	pH	5.03	4.04	4.03
5	Color	Thin green spots	Thin green spots	Thin green spots
	Aroma	Acidic alcohol	Acidic alcohol	Acidic alcohol
	pH	5.00	4.03	4.00
7	Color	Thick green spots	Thick green spots	Thick green spots
	Aroma	Sharp alcohol	Sharp alcohol	Sharp alcohol
	pH	4.05	3.08	3.07
9	Color	Thin green spots	Thin green spots	Thin green spots
	Aroma	Methionine acid	Methionine acid	Methionine acid
	pH	4.04	4.05	4.06

Table 3: Identified chemical compounds at various fermentation inoculum levels and fermentation length

Fermentation time (W)	Inoculum level (D)	Key chemical compounds
W1: 3 days	D1: 1×10^7 spores	- 9-Hexadecenoic Acid, Methyl Ester (Z)
		- Ethyl Linoleate
		- Ethyl Oleate
	D2: 2×10^7 spores	- cis-13-Eicosenoic Acid, Methyl Ester
		- Elaidic Acid, Isopropyl Ester
		- Stigmasta-5,22-dien-3-ol, Acetate
	D3: 3×10^7 spores	- Methyl Hexadec-9-enoate
		- Cholest-5-ene, 3-Bromo- (3β)-
		- Cyclopropane Octanoic Acid, 2-Octyl-
W2: 5 days	D1: 1×10^7 spores	- 9-Octadecenoic Acid, Methyl Ester (CA)
		- Hexadecenoic Acid, Ethyl Ester
		- Methyl (11R,12R,13S)-12,13-Epox
	D2: 2×10^7 spores	- gamma-Linolenic Acid, Methyl Ester
		- Stigmast-5-en-3-ol, (3β)-
		- Cholesterol, Pentafluoropropionate
	D3: 3×10^7 spores	- 9-Octadecenoic Acid (Z)-Tetradecyl Ester
		- Methyl Tetradecanoate
		- Chol-7-en-12-ol, (5β.,12α.)-
W3: 7 days	D1: 1×10^7 spores	- Methane, Oxybis-Dimethyl Ether
		- Isoamyl Laurate
		- Methyl (11R,12R,13S)-12,13-Epox
	D2: 2×10^7 spores	- cis-11-Eicosenoic Acid, Methyl Ester

		- Stigmast-5-en-3-ol, Oleate
		- Cholest-5-ene, 3-Bromo-, (3β)-
	D3: 3×10^7 spores	- 9-Octadecenoic Acid (Z), Methyl Ester
		- Methyl Tetradecanoate
		- Cholest-5-ene, 3-Bromo-, (3β)-
W4: 9 days	D1: 1×10^7 spores	- Pentadecenoic Acid, Methyl Ester
		- Phytol Isomer
		- Stigmast-5-en-3-ol, Oleate
	D2: 2×10^7 spores	- 9,12-Octadecenic Acid (ZZ)-met
		- Cyclopropane Octanoic Acid, 2-Octyl-
		- Tricosanoic Acid, Methyl Ester
	D3: 3×10^7 spores	- Chol-7-en-12-ol, (5β.,12α.)-
		- Cholest-5-ene, 3-Bromo-, (3β)-
		- Stigmast-5-en-3-ol, (3β)-

D: Inoculum level of Saccharomyces cerevisiae (D1: 1×10^7 spores; D2: 2×10^7 spores; D3: 3×10^7 spores); W: Fermentation Time (W1: 3 days; W2: 5 days; W3: 7 days; W4: 9 days)

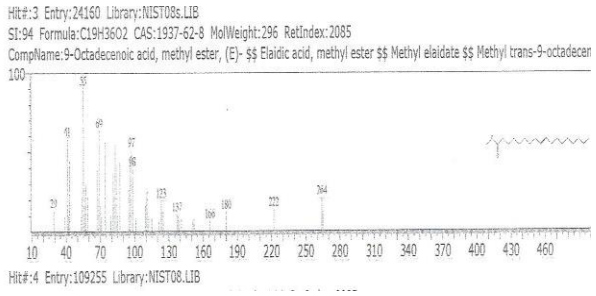

Fig 1: Mass Spectrum of 9-Octadecenoic Acid, Methyl Ester. The spectrum shows the mass-to-charge ratio (m/z) of ionized fragments with notable peaks at m/z 29, 41, 55, 69, 97, 123, 180, 222, and 264, that were identified in the feed treatment.

Biological Quality

The biological quality of the fermented substrate was assessed by measuring the Serum Alkaline Phosphatase (ALP) and Thyroxine (T4) levels in ducks fed with the supplemented feed. The optimal fermentation conditions (3×10^7 spores/100 g for nine days) resulted in a significant increase in ALP and T4 levels, suggesting enhanced enzyme activity and thyroid function in ducks (Table 4). These findings indicate that the fermented substrate successfully enhanced the bioavailability of Zn and Cu, contributing to improved metabolic processes in the birds.

Average Zn Content

The results indicated a significant interaction effect between inoculum levels and fermentation time on Zn content in the substrate ($p<0.01$). This suggests that the effect of inoculum levels on Zn content varied depending on the fermentation time. The highest Zn content was observed with a nine-day fermentation period at the highest inoculum level (3×10^7 spores/100 g), attributed to the optimal growth and activity of *Saccharomyces cerevisiae*, which facilitated the conversion of Zn into a more bioavailable protein-bound form (Teng *et al*., 2017).

Average Cu Content

Similarly, the Cu content in the substrate was significantly influenced by the interaction between fermentation time and inoculum levels ($p<0.01$). The highest Cu content was achieved after nine days of fermentation with the highest inoculum level, reflecting the bioconversion of Cu into a more bioavailable form, as facilitated by *Saccharomyces cerevisiae* (Teng *et al*., 2017).

The Average Content of Serum Alkaline Phosphatase

Fermentation time (W) had a significant effect on ALP levels ($p<0.01$), with the highest ALP levels observed after nine days of fermentation. There was no significant interaction between inoculum levels and fermentation time so the fermentation time independently affects ALP levels significantly. The increase in ALP is directly related to the higher Zn content in the substrate, as Zn is a known cofactor for ALP, suggesting that the biosynthesized Zn was efficiently utilized by the ducks (Setiyatwan, 2007).

The Average Content of Serum Thyroxine (T4)

The statistical analysis revealed significant effects of both inoculum levels (D) and fermentation time (W) on T4 levels ($p<0.05$). This indicates that both the amount of inoculum and the duration of fermentation contributed to the observed changes in T4 levels independently. The highest T4 levels were achieved with the optimal conditions of 3×10^7 spores/100 g substrate and nine days of fermentation. This suggests that the balanced bioavailability of Zn and Cu in the substrate, which is critical for thyroid function, was successfully optimized through these fermentation conditions (Setiyatwan, 2007).

Table 4: Average Content of Zn, Cu, ALP, and T4 in 9-octadecenoic acid, methyl ester substrate after fermentation for each treatment combination

	Inoculum Levels (D)[1]			Fermentation length (W)[2]				p-value		
Parameter	1.107	2.107	3.107	3	5	7	9	D	W	D*W
Zn (mg/kg)	79.3^{a}±1.1	80.12^{ab}±1.5	80.9^{b}±1.7	81.65^{b}±1.8	79.76^{a}±1.4	79.0^{a}±1.2	80.0^{a}±1.4	0.02	0.02	0.01
Cu (mg/kg)	429.7^{a}±12	435.2^{b}±15	434.41^{ab}±17	437.01^{bc}±16	436.07^{b}±15	427.79^{a}±14	431.55^{b}±15	0.04	0.01	0.01
Plasma ALP (U/L)	85.13±2.1	89.25±2.2	100.40±2.8	75.3^{a}±1.9	85.67^{a}±2.0	93.33^{ab}±2.0	112^{b}±2.9	0.07	0.01	0.1
Plasma T4 (mg/dl)	0.58^{b} ±0.01	0.46^{a}±0.01	0.69^{c}±0.02	0.42^{a}±0.01	0.52^{ab}±0.01	0.67^{ab}±0.02	0.7^{b}±0.02	0.03	0.02	0.23

[1]spores/100 g; [2]day; Different letters in the same column indicate significant differences

Table 5: Average consumption, weight gain, feed conversion, meat cholesterol, meat protein content, and water holding capacity in ducks fed with rations supplemented with organic acid 9-octadecenoic acid, methyl ester

	Treatment[1]				
Parameter	P0	P1	P2	P3	P4
Feed Consumption (g)	3038,86	3031,67	2997,92	3007,05	3008,61
Weight Gain (g)	1005,83	1102,83	1166,25	1052,92	1059,17
Feed Conversion Ratio	3,02	2,75	2,58	2,84	2,84
Meat Cholesterol (mg/dL)	0,029	0,122	0,019	0,012	0,0335
Meat Protein Content (%)	18,065	17,85	17,5975	17,625	17,1725
Water Holding Capacity (%)	68,375	68,0475	69,895	69,7	73,7725

[1]P0: Basal Ration; P1: Basal Ration +50 ppm Organic Acid 9-Octadecenoic Acid, Methyl Ester; P2: Basal Ration +100 ppm Organic Acid 9-Octadecenoic Acid, Methyl Ester; P3: Basal Ration +150 ppm Organic Acid 9-Octadecenoic Acid, Methyl Ester; P4: Basal Ration +200 ppm Organic Acid 9-Octadecenoic Acid, Methyl Ester; Note: Different letters in the same column indicate significant differences ($p<0.05$)

Experiment 2: Effects of Supplemented Feed on Duck Performance and Meat Quality

This section presents the results of the second phase of the experiment, which focused on testing the use of 9-octadecenoic acid, and methyl ester organic acid in feed on the performance and meat quality of ducks. The experiment aimed to evaluate the effects of incorporating this organic acid into the feed on various performance and meat quality parameters.

Feed Consumption

The supplementation of 9-octadecenoic acid, methyl ester in duck feed significantly influenced feed consumption, with the optimal reduction observed at the 100 ppm supplementation level (P2) (Table 5). The quadratic relationship ($y = 0.0017x^2 - 0.5154x + 3042.5$, $R^2 = 80.32\%$) (Fig. 2) suggests that the ducks' energy needs were met more efficiently, leading to reduced feed intake (Kompiang *et al*., 2001), stated that energy in feed is a determining factor in the amount of feed consumption because livestock consume feed to meet their energy needs. Supplementation of 100 ppm has a balanced amino acid content, making it efficient in digesting feed and absorbing energy, resulting in efficient consumption.

This is in line with the opinion of Selle and Liu (2019) who stated that the balance of amino acids in feed can affect digestibility and energy absorption. Methionine is glycogenic and can increase glucose and glycogen formation, thereby increasing energy digestibility and impacting consumption efficiency. Poultry can utilize energy from methionine amino acids added to feed. Methionine amino acids will undergo deamination and transmethylation to produce propionyl coenzyme A. Propionyl coenzyme A will enter the Krebs cycle to produce carbohydrates. Amino acid balance will also affect energy absorption. Zn mineral is a cofactor in more than 70 types of enzymes (Berdanier *et al*., 2008). These enzymes play a major role in metabolic processes. Zn plays a major role in carbohydrate metabolism, protein synthesis, and nucleic acid metabolism. The cu mineral can improve growth rate and feed utilization efficiency in broiler chickens (Joshua *et al*., 2016). The availability of Zn, Cu, and Methionine in metabolic processes increases feed utilization efficiency. The optimum point is at a usage level of 100 ppm. This indicates the usage level that produces a balance of methionine amino acids, Zn and Cu as well as other nutrients in the feed resulting in efficient feed consumption.

Body Weight Gain

The body weight gain of ducks was significantly affected by the supplementation, with the highest gain recorded at the 100 ppm level (P2) ($y = -0.0102x^2 + 2.1606x + 1014.9$, $R^2 = 65.02\%$) (Fig. 2). This increase in body weight gain is attributed to enhanced nutrient absorption and mineral bioavailability, which positively impacted growth enzyme activity and overall health (Setiyatwan, 2007). The quadratic pattern observed suggests an optimal level of supplementation, beyond which the benefits plateau or decline.

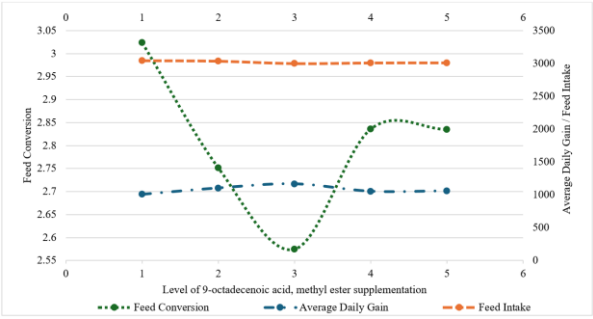

Fig. 2: Quadratic curve of feed consumption, weight gain, and feed conversion in ducks given feed supplemented with 9-octadecenoic acid, methyl ester

The orthogonal polynomial test results show that the treatment effect is significantly different ($p<0.05$) on the increase in duck body weight gain at the second degree (quadratic) of the orthogonal polynomial with the line equation $y = -0.0102x^2+2.1606x+1014.9$ ($R^2 = 65.02\%$). The optimum point for the highest duck body weight gain is obtained from treatment P2, which is feed supplemented with 9-octadecenoic acid, methyl ester organic acid as much as 100 ppm.

Duck body weight gain follows a quadratic pattern, tending to increase to a certain optimum point and then decrease again. The tendency for duck body weight gain to increase when given feed supplemented with 9-octadecenoic acid, methyl ester organic acid is thought to be due to increased absorption, nutrient availability, and bioavailability of minerals, thus having a positive impact on increasing growth enzyme activity and immunity. Amino acids play a role in building body tissues and promoting growth. Increased availability of amino acids will increase growth and final body weight becomes larger. Setiyatwan (2007) stated that supplementation of phytase, ZnO, and $CuSO_4$ into feed can increase the bioavailability of Zn. This condition influences growth enzyme activity, thus increasing body weight. Increased growth is due to the occurrence of mineral homeostasis through the regulation of absorption and excretion between Zn and Cu minerals in the small intestine. Zn and Cu minerals share a transport channel for absorption so that the availability of Zn and Cu for livestock bodies is in accordance with needs.

The optimum point occurs at the level of use of feed supplemented with 9-octadecenoic acid, and methyl ester organic acid as much as 100 ppm. The optimum point of using feed supplemented with 9-octadecenoic acid, methyl ester organic acid as much as 100 ppm indicates that this level is the peak point of supplementation of 9-octadecenoic acid, methyl ester organic acid to produce optimal duck body weight gain. The tendency for duck body weight gain to increase is thought to be due to the balanced availability of Zn and Cu minerals in occupying positions in proteins. Zinc has an affinity that does not compete with Cu minerals so its presence can increase the balance of amino acids. The availability of methionine amino acids, Zn and Cu according to needs has a positive effect on growth.

The addition of 9-octadecenoic acid organic acid supplementation to the feed will increase the content of methionine, Zn, and Cu in the feed. The optimum point of 9-octadecenoic acid organic acid supplementation is 100 ppm in treatment P2. Livestock growth is influenced by protein intake from feed (Suci *et al*., 2005). The quality of protein from feed is determined by the content of essential amino acids with a good balance. Essential amino acids must be present in feed ingredients because they cannot be synthesized in the body of livestock. Jankowski *et al*. (2014) stated that methionine is an essential amino acid for poultry. Fleck and Petrosyan (2014) stated that the amino acid methionine is one of the frameworks for forming body proteins. The amino acid methionine is very necessary for growth rate (Wu, 2014). Methionine is a sulfur donor for cysteine and cystine. Cysteine gets sulfur from methionine and carbon skeleton from serine. Methionine administration needs to pay attention to protein levels, physical form, and palatability of feed ingredients. This is because the amino acid methionine is a toxic amino acid if given excessively. Excessive administration can reduce consumption and growth rates (Son *et al*., 2024). This is suspected to be one reason for the occurrence of an optimum point of 100 ppm then tends to decrease after P2 because the need for methionine has been met at P2. The methionine content in P4 and P5 is suspected to exceed the amount needed by ducks.

Feed Conversion

The efficiency of feed conversion improved significantly with the 100 ppm supplementation, achieving the best FCR of 2.58 (P2) ($y = 0.00005x^2 - 0.0062x+3.0032$, $R^2 = 73.19\%$) (Fig. 2). The improvement in FCR is likely due to the balanced provision of methionine, Zn, and Cu, which enhanced the ducks' metabolic efficiency (Bunchasak, 2009). The results indicate that the 100 ppm supplementation level is optimal for achieving the best feed conversion efficiency.

The results of orthogonal polynomial tests show that the treatment effect is significantly different ($p<0.05$) on increasing the efficiency of duck feed conversion at the second degree (quadratic) of an orthogonal polynomial with a line equation $y = 0.00005x^2 - 0.0062x+3.0032$ ($R^2 = 73.19\%$). The highest optimum point of duck weight gain was obtained from treatment P2, which was feed supplemented with 9-octadecenoic acid, methyl ester organic acid as much as 100 ppm. The administration of methionine, Zn, and Cu in the form of 9-octadecenoic acid, methyl ester organic acid is a balanced organic administration so it is expected that there will be no poisoning from each part. Duck feed conversion given

feed supplemented with 9-octadecenoic acid, methyl ester organic acid follows a quadratic pattern, which tends to be more efficient until a certain optimum point then decreases again. The tendency for more efficient conversion of duck feed given feed supplemented with 9-octadecenoic acid, and methyl ester organic acid is suspected due to sufficient intake of methionine amino acids, Zn, and Cu minerals. This is suspected that sufficient methionine amino acids can increase energy availability and increase growth. The same thing happens to more efficient use of feed (Bunchasak, 2009). The optimum point occurs at the level of use of feed supplemented with 9-octadecenoic acid, and methyl ester organic acid as much as 100 ppm. The amount of 9-octadecenoic acid, methyl ester organic acid at 100 ppm shows that this level is the peak point of supplementation with 9-octadecenoic acid, methyl ester organic acid to produce optimal duck feed conversion. Duck feed efficiency tends to be more efficient due to sufficient need for methionine amino acids, The optimum point of supplementation with 9-octadecenoic acid, methyl ester organic acid is at P2 at 100 ppm. This is suspected to be one reason for the occurrence of an optimum point of 100 ppm then tends to be less efficient after P2 because the need for methionine has been met at P2. The methionine content in P4 and P5 is suspected to exceed the amount needed by ducks.

Cholesterol Content of Duck Meat

The meat cholesterol content was significantly reduced by the supplementation, with the lowest level observed at the 150 ppm supplementation (P3) ($y = -0.006x^2+0.000000005x+0.0566$, $R^2 = 14.6\%$) (Fig. 3). This reduction is attributed to the enhanced lipid metabolism facilitated by the methionine, Zn, and Cu in the feed, which promoted the beta-oxidation of fatty acids and reduced fat deposition in the ducks (Zheng *et al*., 2012). The quadratic relationship suggests that there is an optimal supplementation level for achieving the lowest cholesterol content in meat.

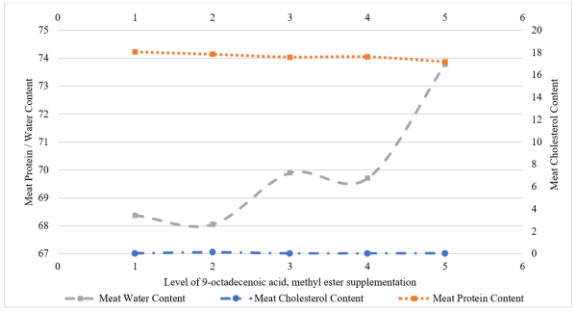

Fig. 3: Quadratic curves of meat cholesterol content, protein content, and water holding capacity in ducks fed with 9-octadecenoic acid, methyl ester supplemented rations

The results of orthogonal polynomial tests show that the treatment effect is significantly different ($p<0.05$) on decreasing the cholesterol content of duck meat at the second degree (quadratic) of an orthogonal polynomial with a line equation $y = -0.006x^2 +0.000000005x+0.0566$ ($R^2 = 14.6\%$). The lowest point of duck meat cholesterol content was obtained from treatment P3, which was fed supplemented with 9-octadecenoic acid, methyl ester organic acid as much as 150 ppm. Fattening in the body comes from the feed given and the fat-forming genes from the duck itself. As age increases, body fat content increases. High fat in the body will cause an increase in LDL levels, which is a lipoprotein rich in cholesterol (Dietschy, 1997). The more fat that is excreted by the body will result in a decrease in cholesterol content in the body (Hu *et al*., 2019). Beta oxidation of fatty acids can reduce fat deposits in the form of cholesterol, triglycerides, bile salts, and steroid hormones. Beta oxidation of fatty acids can be increased by carnitine compounds as carriers of long-chain fatty acids. Carnitine can be synthesized in the body with macronutrient compounds methionine, lysine, and micronutrient compounds such as niacin, $FeSO_4$, Pyridoxin, and Ascorbic Acid, with the help of specific enzymes. Fatty acid coenzyme A (Acetyl Co-A) formed in the cytoplasm is carried into the mitochondria with the help of carnitine molecules. In mitochondria, there is degradation of fatty acids, Asil CoA will be passed into the Krebs cycle and carnitine molecules will be released back into the cytoplasm. The administration of methionine in the form of 9-octadecenoic acid, methyl ester organic acid is an organic administration. The administration of methionine bound to Zn and Cu needs to pay attention to the balance level of lysine amino acids. Methionine given in treatments P2 and P3 is the best administration. This shows that giving methionine in the form of 9-octadecenoic acid, and methyl ester organic acid as much as 100 and 150 ppm effectively reduces duck meat cholesterol content. The decrease in meat cholesterol is also supported by an increase in alkaline phosphatase enzyme activity, which regulates phosphorylation processes related to energy metabolism (Zhuang *et al*., 2022). Ceruloplasmin is an enzyme that plays a role in the absorption and transport of Fe needed for hemoglobin synthesis. Beta oxidation of fatty acids, alkaline phosphatase enzyme, and ceruloplasmin are activated by their respective precursors by methionine amino acids, Zn minerals, and Cu minerals.

The cholesterol content of duck meat given feed supplemented with 9-octadecenoic acid, methyl ester organic acid feed supplement follows a quadratic pattern, which tends to decrease until the lowest point and then increases again. Increasing the use of 9-octadecenoic acid, methyl ester organic acid Feed Supplement as a source of methionine amino acids, Zn and Cu minerals reduces the cholesterol content of duck meat up to a usage limit of 150

ppm. This shows a decrease in cholesterol content and the achievement of a minimum point. The optimum point occurs at the level of use of 9-octadecenoic acid, methyl ester organic acid Feed Supplement at 150 ppm ((150; 0.012)). The optimum level of use (150; 0.012) of 9-octadecenoic acid, methyl ester organic acid Feed Supplement at 150 ppm shows that at this level it is the peak point of using 9-octadecenoic acid, methyl ester organic acid Feed Supplement that can produce the lowest cholesterol content in meat. Supplementation of 9-octadecenoic acid, methyl ester organic acid Feed Supplement in duck feed increases the availability of methionine amino acids, which also has a positive effect on the availability of other amino acids such as threonine, valine, and lysine. These amino acids act as body tissue builders and play a role in growth. Zn from 9-octadecenoic acid, methyl ester organic acid increases its availability as a result of increased Zn absorption. Zinc is a cofactor in more than 70 types of enzymes (Berdanier *et al*., 2008). These enzymes are largely involved in metabolic processes and are important for maintaining the stability and integrity of bio membranes. As part of the enzyme system, Zn plays a major role in carbohydrate metabolism, protein synthesis, and nucleic acid metabolism (Dale, 1994). Zinc can stimulate growth, improve performance, and improve carcass quality.

Protein Content of Duck Meat

The protein content of duck meat showed a significant decrease at the 100 ppm supplementation level (P2) ($y = -6E\text{-}06x^2 - 0.0029x+18.036$, $R^2 = 91.83\%$) (Fig. 3). This decrease is suspected to be due to the increased use of amino acids for carnitine formation rather than protein synthesis, leading to lower meat protein content. The results suggest that while 100 ppm is optimal for other parameters, it may not be ideal for maintaining the highest protein content in duck meat.

The results of orthogonal polynomial tests show that the treatment effect is significantly different ($p<0.05$) on decreasing the protein content of duck meat at the second degree (quadratic) of an orthogonal polynomial with a line equation $y = -6E\text{-}06x^2 - 0.0029x+18.036$ ($R^2 = 91.83\%$). The lowest point of duck meat protein content was obtained from treatment P2, which was feed supplemented with 9-octadecenoic acid, methyl ester organic acid as much as 100 ppm.

The tendency for the protein content of duck meat given feed supplemented with 9-octadecenoic acid, and methyl ester organic acid to decrease is suspected due to increased absorption, nutrient availability, and biological availability of minerals, which has a positive impact on increasing the work of growth and immune system enzymes. Amino acids that play a role in body tissue composition, namely methionine and lysine amino acids in such conditions are widely used for the formation of carnitine, thus reducing the protein content of meat. The protein content of duck meat follows a quadratic pattern, which tends to decrease until a certain low point. The tendency for the protein content of duck meat given feed supplemented with 9-octadecenoic acid, and methyl ester organic acid to decrease is suspected because methionine and lysine amino acids are used for the formation of carnitine. Supplementing 9-octadecenoic acid, methyl ester organic acid in duck feed increases the availability of methionine amino acids, which also has a positive effect on the availability of other amino acids such as threonine, valine, and lysine. These amino acids act as body tissue builders. Zn from 9-octadecenoic acid, methyl ester organic acid increases its availability because of increased Zn absorption. As part of the enzyme system, Zn plays a major role in carbohydrate metabolism, protein synthesis, and nucleic acid metabolism (Dale, 1994). Zinc can stimulate growth, improve performance, and improve carcass quality.

Water Holding Capacity of Duck Meat

The Water-Holding Capacity (WHC) of duck meat was significantly enhanced by the supplementation, with the highest WHC observed at the 50 ppm supplementation level (P1) ($y = 0.0002x^2 - 0.0137x+68.434$, $R^2 = 90.27\%$) (Fig. 3). The improved WHC is likely due to the better integrity of meat proteins, which was facilitated by the balanced mineral and amino acid content in the feed. The results suggest that lower supplementation levels may be more effective for improving WHC, potentially due to the specific interaction between the minerals and meat proteins.

The results of orthogonal polynomial tests show that the treatment effect is significantly different ($p<0.05$) on increasing the water-holding capacity of duck meat at the second degree (quadratic) of an orthogonal polynomial with a line equation $y = 0.0002x^2 - 0.0137x+68.434$ ($R^2 = 90.27\%$). The lowest point of duck meat water holding capacity was obtained from treatment P1, which was feed supplemented with 9-octadecenoic acid, methyl ester organic acid as much as 50 ppm.

Duck meat water holding capacity follows a quadratic pattern, which tends to increase until a certain point. Water Holding Capacity is an indicator to measure the ability of meat to bind water that is added while there is an external force effect. Good meat protein integrity causes an increase in the ability of meat to hold water and vice versa. The higher the water that comes out, the lower its binding power. Increasing the use of 9-octadecenoic acid, methyl ester organic acid feed supplement increases water holding capacity. This shows that the quality of the resulting meat is getting better.

While our findings clearly highlight the benefits of incorporating biosynthesized Zn, Cu, methionine, and 9-octadecenoic acid methyl ester into duck feed, several

important limitations should be acknowledged. First, because we only worked with unsexed ducks, we could not determine whether responses differed between males and females. Additionally, our study took place under controlled conditions that may not mirror the full range of commercial production environments. External factors like seasonal shifts, genetic diversity, and varied management practices could influence the consistency and extent of these observed effects. Future research that includes sex-specific analyses and trials under more diverse rearing conditions will help us better understand how these supplements perform in real-world settings.

In practical terms, establishing the right supplementation levels can help poultry producers formulate cost-effective, nutritionally balanced diets that improve feed efficiency, growth rates, and meat quality. The observed enhancements in enzyme activity, metabolic function, and overall performance suggest that these supplements can be strategically integrated to boost production outcomes, maintain animal health, and ensure a higher-quality final product. As the industry continues to seek sustainable and efficient feeding strategies, our results can inform decision-making and support the adoption of tailored supplementation programs that ultimately enhance both productivity and profitability.

Conclusion

The biosynthesis of Zn, Cu, and methionine substrates using *Saccharomyces cerevisiae* as much as 3.10^7 spores/100 g of substrate and a nine-day period is the best treatment combination. In this treatment, 9-octadecenoic acid, methyl ester organic acid with a molecular weight of 296 is formed. This organic acid is a short-chain straight-form organic acid, so ionization is easy and it is the most stable bond. In this treatment, the Zn content increased from 76.73-84.60 mg/kg. The Cu content increased from 422.18-447.04 mg/kg. The serum alkaline phosphatase content increased from 64.00-124.00 U/l and the serum thyroxine content increased from 0.35-1.00 ug/dl.

The optimal supplementation level identified was 100 ppm of 9-octadecenoic acid, methyl ester, which led to significant improvements in feed conversion efficiency, body weight gain, and water-holding capacity of the meat. However, it also resulted in a slight reduction in meat protein content, indicating a potential trade-off that requires further investigation.

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Author's Contributions

Hendi Setiyatwan: Conceptualization, methodology, formal analysis, investigation, resources, data curation, writing of the original draft, project administration, and funding acquisition.

Muhammad Rifqi Ismiraj: Methodology, formal analysis, investigation, data curation, writing of the original draft, and review and editing of the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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Supplementary

Table S1: The complete list of identified compounds in this study

Fermentation length	Inoculum level		
	D1	D2	D3
W1	Tetradecenoic Acid, methyl ester (CAS); Pentadecenoic acid, methyl ester; 9-Hexadecenoic acid, methyl ester, (Z; Hexadecenoic acid, methyl ester, (CAS) E; Heptadecanoic acid, methyl ester; Methyl 10-trans,12-cis-octadecadienoa; 9- octadecenoic acid, methyl ester (CA; octadecenoic acid, methyl ester; Ethyl Linoleate; Ethyl Oleate; 1e, 4a-DIHYDROXY-(DELTA.6,7)-trans; Methyl 9. cis., 11. trans.t,13. trans-octa; cis-13-Eicosenoic acid, methyl ester; Eicosenoic acid, methyl ester (CAS) Ar; n-Propyl 9, 12- octadecadienoate; 9- octadecenoic acid (Z)-(tetrahydro-; 2H-Pyran-2-one, tetrahydro-6-tridecyl-; Elaidic acid, isopropyl ester (CAS) M; BENZOL, 1,2-BIS (9-BORABICYCLO (3,3; Docosanoic acid, methyl ester (CAS) M; Tricosanoic acid, methyl ester; Stigmasta-5,22-dien-3-ol, acetate, (3-; octadecanoic acid, ethyl ester; Stigma-5-en-3-ol,oleate; Cholest-5-ene, 3-bromo-, (3.beta)-;	Pentadecenoic acid, methyl ester; 9-Hexadecenoic acid, methyl ester, (Z; Methyl 10-trans,12-cis-octadecadienoa; 9- octadecenoic acid, methyl ester (CA; octadecenoic acid, methyl ester; Ethyl Linoleate; Ethyl Oleate; cis-13-Eicosenoic acid, methyl ester; Eicosenoic acid, methyl ester (CAS) Ar; Hexacosanoic acid, methyl ester; Methyl Hexadec-9-enoate; Hexadecenoic acid, ethyl ester, (CAS) E; cis-10-Heptadecanoic acid, methyl ester; 9,12- octadecenoic acid, (ZZ)-,met; Cyclopropane 0ctanoic acid,2-octyl-,m; Docosanoic acid, methyl ester (CAS) M; Sandaracopimaradiene; Heptadecanoic acid, methyl ester; pentacosanoic acid ,methyl ester; hexacosanoic acid ,methyl ester; Cholesterol, pentafluoropropionate; Stigmasta-5,22-dien-3-ol, acetate, (3-; octacosanoic acid,methyl ester (CAS); hexanoic acid ,methyl ester (CAS) met;	Pentadecenoic acid, methyl ester; 9-Hexadecenoic acid, methyl ester, (Z; Hexadecenoic acid, methyl ester, (CAS) E; Heptadecanoic acid, methyl ester; Methyl 10-trans,12-cis-octadecadienoa; octadecenoic acid, methyl ester; Ethyl Linoleate; Ethyl Oleate; Methyl (11R,12R, 13S)-(Z)-12-13-epox; Isoamyl laurate; Methyl 9. cis., 11. trans.t,13. trans-octa cis-13-Eicosenoic acid, methyl ester; Eicosenoic acid, methyl ester (CAS) Ar; Hexacosanoic acid, methyl ester; Methyl Hexadec-9-enoate; cis-10-Heptadecanoic acid, methyl ester; octadecenoic acid, methyl ester; Ethyl Linoleate; Ethyl Oleate; Methyl (11R,12R, 13S)-(Z)-12-13-epox; Methyl 9. cis., 11. trans.t,13. trans-octa; cis-13-Eicosenoic acid, methyl ester;

		9- octadecenoic acid, methyl ester (E); Hexadecanoic acid, methyl ester; Hexadecanoic acid, ethyl ester (CAS) E;	Eicosenoic acid, methyl ester (CAS) Ar; Hexacosanoic acid, methyl ester; Methyl Hexadec-9-enoate; cis-10-Heptadecanoic acid, methyl ester; Docosanoic acid, methyl ester (CAS) M; Tricosanoic acid, methyl ester; 9- octadecenoic acid (Z)-, tetradecyl es; Nonanal dimethyl acetat; Nonanal (CAS) n-nonanal; Nonanoic acid, 9-oxo-, methyl ester; Methyl myristoleate; Tetradecanoic acid, methyl ester (CAS; FUMARSAEURE, ETHYLESTER,2-(2-ME
W2	9- octadecenoic acid, methyl ester (CA octadecenoic acid, methyl ester cis-13-Eicosenoic acid, methyl ester Eicosenoic acid, methyl ester (CAS) Ar Methyl Hexadec-9-enoate cis-10-Heptadecanoic acid, methyl ester 9,12- octadecenoic acid, (ZZ)-,met Docosanoic acid, methyl ester (CAS) M Tricosanoic acid, methyl ester Tetradecenoic Acid, methyl ester 9- octadecenoic acid (Z), methyl ester9,19-cyclolanostan-3-ol,24-methylene Hexadecenoic acid, ethyl ester, (CAS) Heptadecanoic acid, methyl ester, (CAS) 5-octadecenoic acid, methyl ester	9- octadecenoic acid, methyl ester (CA octadecenoic acid, methyl ester Methyl 9. cis., 11. trans.t,13. trans-octa cis-13-Eicosenoic acid, methyl ester Eicosenoic acid, methyl ester (CAS) Ar Methyl Hexadec-9-enoate Docosanoic acid, methyl ester (CAS) M stigmast-4-en-3-one(CAS)4-stigmas 9,19-cyclolanostan-3-ol,24-methylene Hexadecenoic acid, ethyl ester, (CAS) 9- octadecenoic acid (Z)-tetradecyl es hexanoic acid ,methyl ester (CAS) met 5-octadecenoic acid, methyl ester Choles- 5-ene, 3- bromo-, (3 beta)- 9,12-Octadecadienoic acid (Z,Z)-,met Stigmast-5-en-3-ol,(3.beta.)- (CAS) 2 Hexadecanoic acid, methyl ester pentacosanoic acid ,methyl ester	octadecenoic acid, methyl ester Ethyl Linoleate n-Propyl 9, 12- octadecadienoate Tetradecenoic Acid, methyl ester octacosanoic acid,methyl ester 9- octadecenoic acid (Z), methyl ester 9- octadecenoic acid (Z)-tetradecyl es hexanoic acid ,methyl ester (CAS) met 5-octadecenoic acid, methyl ester Hexadecanoic acid, methyl ester (CAS) 9,12-Octadecadienoic acid (Z,Z)-,met Stigmast-5-en-3-ol,(3.beta.)- (CAS) 2 Cholest-5-ene, 3-bromo-, (3.beta)- Chol-7-en-12-ol,(5 beta.,12.alpha.)-
W3	Methane, oxybis-(CAS) Dimethyl ether Tetradecenoic Acid, methyl ester (CAS) Pentadecenoic acid, methyl ester 9-Hexadecenoic acid, methyl ester, (Z 9-Hexadecenoic acid, methyl ester, (Z Hexadecenoic acid, methyl ester, (CAS) Methyl 10-trans,12-cis-octadecadienoa 9- octadecenoic acid, methyl ester (CA octadecenoic acid, methyl ester Ethyl Linoleate Ethyl Oleate Methyl (11R,12R, 13S)-(Z)-12-13-epox Isoamyl laurate cis-13-Eicosenoic acid, methyl ester Eicosenoic acid, methyl ester (CAS) Ar Methyl Hexadec-9-enoate cis-10-Heptadecanoic acid, methyl ester ,12- octadecenoic acid, (ZZ)-,met Docosanoic acid, methyl ester (CAS) M Tricosanoic acid, methyl ester Tetradecenoic Acid, methyl ester 3-cyano-3-octyl-1,4-cyclohexadiene methyl tetradecanoate Butyl 9,12-octadecadienoate Stigmasta-5,22-dien-3-ol, acetate, (3-	hexacosanoic acid ,methyl ester Methyl 10-trans,12-cis-octadecadienoa 9- octadecenoic acid, methyl ester (CA octadecenoic acid, methyl ester Ethyl Linoleate Ethyl Oleate Methyl (11R,12R, 13S)-(Z)-12-13-epox Isoamyl laurate cis-13-Eicosenoic acid, methyl ester Eicosenoic acid, methyl ester (CAS) Ar Hexacosanoic acid, methyl ester Methyl Hexadec-9-enoate cis-10-Heptadecanoic acid, methyl ester 9,12- octadecenoic acid, (ZZ)-,met Docosanoic acid, methyl ester (CAS) M Tricosanoic acid, methyl ester Tetradecenoic Acid, methyl ester 2,6,10,14,18,22-tetracosahexaene,2, Colest-5-ene, 3-bromo-, (3beta.)- Stigmast-5-en-3-ol, oleate 9- octadecenoic acid (Z)-tetradecyl es hexacosanoic acid ,methyl ester Stigmasta-5,22-dien-3-ol, acetate, (3- octacosanoic acid,methyl ester (CAS) 5-octadecenoic acid, methyl ester 2-Tertbutyl cyclohexyl isopropylphospat 2-Hexadecen-1-0l, 3,7,11,15-tetramet tetracosanoic acid, methyl ester 9-octadecenoic acid (Z), methyl ester, (E) Hexadecenoic acid, 2-methylpropyl est ester	9-Hexadecenoic acid, methyl ester, (Z Hexadecenoic acid, methyl ester, (CAS) E Heptadecanoic acid, methyl ester Methyl 10-trans,12-cis-octadecadienoa 9- octadecenoic acid, methyl ester (CA octadecenoic acid, methyl ester Ethyl Linoleate Ethyl Oleate Methyl (11R,12R, 13S)-(Z)-12-13-epox Isoamyl laurate 1e, 4a-DIHYDROXY-(DELTA.6,7)-trans Methyl 9. cis., 11. trans.t,13. trans-octa cis-13-Eicosenoic acid, methyl ester Eicosenoic acid, methyl ester (CAS) Ar n-Propyl 9, 12- octadecadienoate 9- octadecenoic acid (Z)-(tetrahydro-2H-Pyran-2-one, tetrahydro-6-tridecyl- Elaidic acid, isopropyl ester (CAS) M BENZOL, 1,2-BIS (9-BORABICYCLO (3,3 Tetracosanoic-3-octyl-1,4-cyclohexadiene Hexacosanoic acid, methyl ester 9- octadecenoic acid (Z)-tetradecyl e Heptadecanoic acid, methyl ester Cholesterol, pentafluoropropionate 2-Tertbutyl cyclohexyl isopropylphospat 2-Hexadecen-1-0l, 3,7,11,15-tetramet Adipic acid, isohexyl methyl ester 2-CYCLOHEXYL-3-ISOPROFYL-PENT-4- Hexanedioic acid, bis(2-ethylhexyl) est
W4	Pentadecenoic acid, methyl ester	9-Hexadecenoic acid, methyl ester, (Z	octadecenoic acid, methyl ester

	9-Hexadecenoic acid, methyl ester, (Z	Methyl 10-trans,12-cis-octadecadienoa	Ethyl Oleate
	Methyl 10-trans,12-cis-octadecadienoa	octadecenoic acid, methyl ester	Eicosenoic acid, methyl ester (CAS) Ar
	9- octadecenoic acid, methyl ester (CA	Ethyl Linoleate	Hexacosanoic acid, methyl ester
	octadecenoic acid, methyl ester	Ethyl Oleate	Methyl Hexadec-9-enoate
	Ethyl Linoleate	Methyl (11R,12R, 13S)-(Z)-12-13-epox	Hexadecenoic acid, ethyl ester, (CAS) E
	Ethyl Oleate	Isoamyl laurate	cis-10-Heptadecanoic acid, methyl ester
	Isoamyl laurate	Eicosenoic acid, methyl ester (CAS) Ar	gamma, -linolenic acid, methyl ester
	Eicosenoic acid, methyl ester (CAS) Ar	Tetradecenoic Acid, methyl ester	9,12- octadecenoic acid, (ZZ)-,met
	Tricosanoic acid, methyl ester	9- octadecenoic acid (Z)-tetradecyl es	Cyclopropane 0ctanoic acid,2-octyl-,m
	Stigmast-5-en-3-ol, oleate	PHYTOL ISOMER	cis-11-Eicosenoic acid, methyl ester
	24(s)-ethyl-3-alpha.,5-alpha,-cyclocho	cis-5-dodecenoic acid, methyl ester	Docosanoic acid, methyl ester (CAS) M
	24-methylenecyclo artan-3-one	Cholesterol, pentafluoropropionate	Tricosanoic acid, methyl ester
	9- octadecenoic acid (Z)-tetradecyl es	Hexadecenoic acid, 2-methylpropyl est	2,6,10,14,18,22-tetracosahexaene,2,
	PHYTOL ISOMER	ester	octacosanoic acid,methyl ester
	cis-5-dodecenoic acid, methyl ester	octadecanoic acid, ethyl ester	9- octadecenoic acid (Z), methyl ester
	2-pentadeconone,6,10,14-trimethyl-	Hexadecanoic acid, methyl ester	methyl tetradecanoate
	hexacosanoic acid ,methyl ester	Hexadecanoic acid, ethyl ester (CAS) E	9- octadecenoic acid (Z)-tetradecyl es
	Hexadecanoic acid, methyl ester	Heptadecenoic acid, methyl ester	Choles- 5-ene, 3- bromo-, (3 beta)-
	Hexadecanoic acid, ethyl ester (CAS) E		Stigma-5-en-3-ol,oleate
			Methyl 18-methylnonadecanoate
			1-(Pent-4-ynyl)pyranol[3,4-b]indol-3-o
			1-phenyl-6-(tert-butyldimethylsilyl)-1,