Effects of live yeast supplementation on serum oxidative stress biomarkers and lactation performance in dairy cows during summer

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ABSTRACT

This study aimed to evaluate the effects of live yeast (*Saccharomyces cerevisiae*) (LY) supplementation on serum oxidative stress biomarkers, antioxidant vitamin levels, and lactation performance in dairy cows during summer. A total of 16 lactating cows weighing 707.5 ± 13.1 kg (mean ± standard error) were enrolled and randomly assigned to either supplemented (n = 8) or control group (n = 8). In the supplemented group, the cows were administered with LY product at 10 g/day per cow from mid-July to mid-September for 8 weeks. The serum levels of derivatives of reactive oxygen metabolites in the supplemented group were lower (*P* < 0.05) at week 6. The serum retinol and blood glucose concentrations in the supplemented group were higher (*P* < 0.01) at week 8. LY supplementation did not affect physiological responses, such as rectal temperature, respiratory rate, protein and cholesterol metabolism, and lactation performance. During the study period, daily average milk yield decreased in both groups. The reduction rates of milk yield in the supplemented and control groups were 17.6 and 20.0%, respectively. These results suggest that LY supplementation may reduce oxidative stress and improve carbohydrate metabolism in lactating dairy cows during summer.

KEY WORDS: cow, live yeast, milk, oxidative stress, summer season
INTRODUCTION

In the United States, annual losses in the year of 2000 to the dairy industry as a result of heat stress ranged from $897 to $1,507 million due to reduced dry matter intake (DMI), reduced reproductive performance, increased incidence of mastitis, and increased mortality rate [34]. Numerous nutritional strategies to reduce heat stress have been widely investigated; however, many require further investigation to identify specific recommendations [8]. Several studies have reported the common benefits of live yeast (Saccharomyces cerevisiae) (LY) supplementation in dairy cows, including improved feed efficiency and performance [6], increased DMI [21], increased milk yield and milk protein [24], and higher ruminal pH [15].

In addition, there have been reports on the effects of LY administration on the performance of dairy cattle under heat stress during summer. Similar to thermal neutral conditions, LY supplementation in dairy cows during hot weather increased DMI, productivity, and feed efficiency [24], increased milk and solids yield [31], and improved milk production and body condition, in addition to minimizing the loss of weight during calving [2]. According to Schingoethe et al. [32], yeast culture can improve the feeding efficiency of dairy cows under heat stress.

Although the mechanism of action of LY on the rumen microbial ecosystem remains unclear, LY supplementation in dairy cows has been shown to decrease redox potential and increase pH in the rumen [15] and maintain healthy fermentation in the rumen accompanied by an increase in the relative occurrence of fibrolytic and lactate-utilizing bacteria [27]. In addition, LY supplementation in dairy cows has been shown to improve the digestibility of crude protein and acid detergent fiber [39]. Yeast culture provides soluble growth factors, which stimulate the growth of ruminal bacteria that utilize lactate and digest cellulose [3].
Meanwhile, it was found that dairy cows show an increase in the levels of oxidative stress after parturition [1], and that ketotic dairy cows experience oxidative stress, which is presumably associated with hyperketonemia and higher plasma non-esterified fatty acids concentrations [17]. A cow with high body condition score (BCS) can experience oxidative stress [26]. It has also been indicated that a high ambient temperature during summer increases oxidative stress in Japanese Black cows [30].

The temperature humidity index (THI) is an environmental indicator of the risk of heat stress, which accounts for the combined effects of environmental temperature and relative humidity. THI has been used to estimate the effects of thermal stress on livestock and has been shown to be more effective in evaluating the environmental effects on dairy cattle than temperature alone [13]. Lactating dairy cows experience heat stress when THI rises above 72 [28]. Therefore, we hypothesized that LY supplementation may mitigate oxidative stress in dairy cows under heat stress. The effects of LY on serum biomarkers of oxidative stress and antioxidants remain to be fully elucidated. The study aims to evaluate the effects of LY supplementation on serum oxidative stress biomarkers, antioxidant vitamin concentrations, and lactation performance in dairy cows during summer.

MATERIALS AND METHODS

Experimental animals: The experiment was conducted from July 19 to September 20, 2016, in an open-walled, tie-stall barn with fans and a sprinkler at a private dairy farm located in Fukuoka prefecture, Japan. A total of 16 lactating Holstein Friesian cows were enrolled from a commercial herd of 35 dairy cows and randomly assigned to either supplemented group (n = 8) or control group (n = 8) on the basis of parity, lactation stage, and milk production at the start of the experiment. The body weight and number of days
post-calving in the supplemented cows were 715.0 ± 22.9 (mean ± standard error, SE) kg and 188.9 ± 25.4 days, respectively. The body weight and number of days post-calving in the control cows were 700.0 ± 13.9 kg and 179.0 ± 34.5 days, respectively. The numbers of supplemented cows with parities of 1, 2 and 3 were 2, 4 and 2 heads, respectively. The numbers of control cows with parities of 1, 2 and 3 were 3, 2 and 3, respectively. The weeks of lactation in individual cows were shown in Fig. 1.

In addition, daily average milk productions at the beginning of the study in the supplemented and control cows were 28.2 ± 2.4 kg and 27.6 ± 1.1 kg, respectively. The cows in both groups were fed a concentrate (14–16 kg/cow/day), alfalfa hay (4 kg), oat hay (4 kg), and wrapped bale silage of Italian ryegrass (8 kg). The concentrate comprised 16.5% crude protein, 2.0% crude fat, 10.0% crude fiber, 10.0% crude ash, 0.7% calcium, 0.4% phosphorus, and 74.0% total digestible nutrients. Feed was offered twice daily at 05:00 and 16:00 hr. The cows in the supplemented group were administered with LY product (ActiSaf®, Phileo Lesaffre Animal Care, Marcq-en-Baroeul, France) at 10 g/day per cow as a top dress at the morning feeding for 8 weeks. The cows were milked twice daily at 06:00 and 17:00 hr. The environmental temperature and humidity at the center of the barn was measured at 13:00 hr using a heat stress meter (TM-9502, Empex Instruments, Inc., Tokyo, Japan). All protocols were approved by the Animal Ethics Committee of the University of Miyazaki (Approval no. 2016-07-27-Z15).

Sample collection: Blood was collected from the jugular vein into three tubes; a tube containing ethylene diamine tetra acetic acid dipotassium salt dihydrate (EDTA-2K), a tube containing sodium fluoride, and a serum tube at 14:00 hr before the administration of LY and at 1, 2, 4, 6, and 8 weeks after the initial administration. The rectal temperature (RT), respiratory rate (RR), and BCS were measured simultaneously with the collection
of blood. A complete blood count was performed on the EDTA-2K anticoagulated blood using an automated hematology analyzer (pocH-100iV, Sysmex Corp., Kobe, Japan). Blood glucose levels were measured in the sodium fluoride anticoagulated blood using Antsense III glucose analyzer (Horiba, Ltd., Kyoto, Japan). The serum samples were separated and then stored at −80°C until derivatives of reactive oxygen metabolites (d-ROMs), biological antioxidant potential (BAP), blood urea nitrogen (BUN), total cholesterol (T-Cho), and vitamin analyses. A dairy herd performance test performed once a month by the Livestock Improvement Association of Japan, Inc. was used to evaluate milk yield and composition.

Measurement of serum d-ROMs, BAP, BUN, and T-Cho: The serum was thawed and then stirred thoroughly with a vortex mixer. The d-ROMs and BAP were measured using a free radical analyzer (FREE carpe diem, Wismerll Co., Ltd., Tokyo, Japan). In addition, an oxidative stress index (OSI) was estimated using the ratio of d-ROMs/BAP multiplied by 100. Celi [4] proposed that when oxidative stress was evaluated using ROMs and BAP, information on the level of oxidative stress is more accurate when ROMs and BAP data are combined, rather than separate. For measurements of serum BUN and T-Cho, a blood biochemistry automatic analyzer (Fuji Dri-Chem 7000V, FUJI FILM Medical Co., Ltd., Tokyo, Japan) was used.

Measurement of vitamin concentration: The serum concentrations of retinol, α-tocopherol, and β-carotene were analyzed using high-performance liquid chromatography according to the method described by Mirzad et al. [23].

Statistical analysis: All statistical analyses were performed using R version 3.3.2. The Holm’s method was used to adjust for multiple comparisons. Two-way repeated measures analysis of variance was used to determine the effects of treatment. A simple effect was examined if a significant interaction was observed. The results are expressed as the mean
± SE. The rate of milk yield reduction was calculated by dividing the level of the reduced quantity by the original quantity. $P < 0.05$ was considered statistically significant.

RESULTS

In the present study, the weekly mean THI values at week 0 (79.71), week 1 (80.42), week 2 (80.71), week 4 (78.28), week 6 (74.57), and week 8 (74.22) were >72, which indicated that the animals were subjected to heat stress during the entire experimental period (Fig. 2). In both groups of cows, the highest RT and RR values were observed at week 4 (Table 1). However, no significant differences in RT, RR, BCS, or hematological values were observed between the two groups. The changes in serum levels of oxidative stress biomarkers are shown in Fig. 3. At week 6, the serum levels of d-ROMs, a biomarker of oxidative stress, were significantly lower ($P < 0.05$) in the cows supplemented with LY (Fig. 3A). There were no significant differences in the serum levels of BAP or OSI between the two groups during the study period (Fig. 3B and 3C). The changes in serum vitamin levels are shown in Fig. 4. The cows supplemented with LY had higher ($P < 0.01$) serum retinol levels and higher ($P < 0.1$) serum β-carotene concentrations compared with those in the control cows at week 8 (Fig. 4A and 4C). In addition, a higher ($P < 0.1$) serum concentration of α-tocopherol was observed in the supplemented cows at week 2 (Fig. 4B). The changes in blood glucose, BUN, and serum T-Cho levels are shown in Fig. 5. The blood glucose levels were significantly higher ($P < 0.01$) in the cows supplemented with LY than those in the control cows at week 8 (Fig. 5A). There were no significant differences in the BUN or serum T-Cho levels between the two groups during the study period (Fig. 5B and 5C). The changes in lactation performance are listed in Table 2. No significant difference in milk yield or milk composition was observed between the two groups. The daily milk yield in both groups
decreased during the study period, and the rates of milk yield reduction at day 63 in the supplemented and control cows were 17.6 and 20.0%, respectively.

DISCUSSION

The beneficial effects of active LY products have been investigated extensively in ruminant animals. The mechanisms of action of yeast additives on ruminal function include the improvement of rumen maturity by favoring microbial establishment, stabilization of ruminal pH and interactions with lactate-metabolizing bacteria, and increase of fiber degradation and interactions with plant-cell wall degrading microorganisms [6]. The addition of yeast culture in a calf starter was shown to enhance DMI and growth and marginally improve rumen development in dairy calves [16]. An increase of ruminal pH, possibly due to the reduction of lactate concentrations, was observed in the rumen fluid of rumen-cannulated steers administered with yeast culture [38]. A decrease in lactate concentration has also been reported in in vitro mixed ruminal microorganism fermentation supplemented with live Saccharomyces cerevisiae cells [18]. In addition, supplementation with yeast culture was shown to increase total and cellulolytic bacterial numbers in the rumen fluid of rumen-cannulated sheep [25].

Callaway and Martin [3] reported that yeast culture provides soluble growth factors (i.e., organic acids, B vitamins, and amino acids), which stimulate the growth of ruminal lactate-utilizing and cellulolytic bacteria. An increase in fibrolytic bacterium, Fibrobacter, was also observed in the rumen fluid of cows supplemented with LY [37]. Other studies have reported that yeast products reduced redox potential in the rumen in lambs [5] and sheep [20], suggesting that LY is capable of oxygen scavenging and provides improved conditions for the growth and activity of anaerobic rumen bacteria, particularly for highly oxygen sensitive species, such as cellulolytic bacteria.
In the present study, the serum levels of d-ROMs in the supplemented cows at week 6 were significantly lower \((P < 0.05)\) compared with those in the control cows, which indicated that LY products may be able to mitigate some of the negative effects associated with heat stress in dairy cattle. Hassan [11] reported that yeast autolysates exhibited antioxidant activity in addition to reducing-power, DPPH \((1, 1\text{-diphenyl-2-picrylhydrazyl})\) radical scavenging, nitric oxide scavenging, hydroxyl radical scavenging, and metal chelating activities. Fakruddin et al. [10] also observed reducing-power, DPPH scavenging activity, nitric oxide scavenging, and hydroxyl radical scavenging activities of yeast extracts and autolysates. The antioxidative effect of yeast autolysates may be due to their contents of antioxidant agents, including glutathione [11], Maillard reaction products [35], sulfur-containing amino acids [36], and polysaccharides [14]. Therefore, the decreased serum levels of d-ROMs observed in the present study may be attributed to intake of components of LY with antioxidant properties. Concerning the beneficial effects of LY to dairy cows under heat stress, Salvati et al. [31] observed a trend for increased plasma niacin content and lowered decreased respiratory frequency, suggesting that LY supplementation may have stimulated the synthesis of niacin in the rumen and improved heat dissipation. Niacin can stimulate skin vasodilation [19] and increase peripheral heat loss in dairy cows [41]. Therefore, reduced oxidative stress through the intake of LY with probable antioxidant properties may be an additional mechanism for alleviating heat load.

In contrast, LY supplementation did not appear to affect the serum levels of BAP or OSI during the study period. The BAP test provides a global measurement of several antioxidants, including uric acid, ascorbic acid, proteins, α-tocopherol, and bilirubin [4]. Plasma BAP might have decreased also as a direct consequence of reactive oxygen metabolites (ROMs) increase [4]. Chauhan et al. [7] reported decreased levels of plasma ROMs and OSI, accompanied by increased plasma levels of BAP, in sheep under heat
stress supplemented with antioxidants, such as vitamin E and selenium. Since significant increase in serum α-tocopherol and β-carotene concentrations were not observed with LY supplementation, no obvious change in serum levels of BAP or OSI may be related in part to the status of these antioxidant vitamins. There were significant differences in serum retinol concentrations at week 0 and 8 and in serum β-carotene concentrations at week 2 between 2 groups, although the reasons for this remain unclear. No significant differences in milk yield or milk composition were observed between the two groups in the present study. The effects of LY can be considered positive factors for the growth of anaerobic bacteria and thus for digestion in the rumen, which in turn stimulates voluntary food intake and the nutrient supply to the animals, thereby promoting animal performance [12]. Similar to our results, neither Shwartz et al. [33] nor Dehghan-Banadaky et al. [9] observed any significant effect of LY supplementation on milk yield in lactating cows under heat stress. In contrast, another study reported that the milk yield and the milk protein yield and percentage increased with supplementation of LY [29]. The reasons for the inconsistencies regarding changes in milk production following LY supplementation among trials remain unclear. The beneficial effects of LY products may depend on the lactation stage [39], environmental condition [32], and diet or animal management [6]. Similar to our study, a previous study showed that LY supplementation did not significantly affect the BCS or RT and tended to reduce respiration frequency in lactating cows during summer [31]. In the present study, the RT and RR in both groups of cows were highest at week 4, suggesting this is when they experienced maximum discomfort; however, LY supplementation did not affect physiological responses. Blood glucose levels were significantly higher \( (P < 0.01) \) in the supplemented cows at week 8. A significant increase [9] and increased trends in plasma glucose concentrations [31] have been previously observed in dairy cows supplemented with LY during hot summer
conditions. Miller-Webster et al. [22] reported that supplementing diets with yeast culture products increased dry matter digestion, total volatile fatty acid (VFA) production, and propionic acid production in continuous cultures. As propionate is the only major VFA that contributes to gluconeogenesis [40], the increased blood glucose levels observed in this study may be due to increased propionate production by LY supplementation. In contrast to our study, Ayad et al. [2] did not observe changes in plasma glucose levels following yeast supplementation, suggesting that the discrepancy in data may be attributed to differences in the nature of the feed in addition to the dose and duration of yeast supplementation.

Our study had some limitations. First, sample size was small and statistical power was below 0.8. A sample size calculation was not conducted in this study, and the number of experimental animals was determined as shown in materials and methods. Although significant differences in serum d-ROMs and blood glucose levels were found at week 6 and 8, respectively, the limited periods for notable changes may be due to a small sample size. Second, each group included various animals in parity and lactation stage. Namely, the weeks of lactation in cows were not equally balanced between 2 groups.

In conclusion, this study suggested that LY supplementation at 10 g/day per cow may alleviate oxidative stress and improve carbohydrate metabolism in lactating dairy cows during summer. However, LY supplementation did not affect physiological responses, such as RT and RR, protein and cholesterol metabolism, and lactation performance. There are numerous reports describing beneficial effects of LY supplementation; therefore, further studies are required to elucidate the influence of the lactation stage of cows and diet composition as well as the dose and duration of LY supplementation.

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Figure Legends

Fig. 1. Weeks of lactation in individual cows enrolled in this study.

Fig. 2. Changes in temperature and humidity index (THI) during the study period. Dashed line represents the value of 72.

Fig. 3. (A) Mean values (± SE) of serum derivatives of reactive oxygen metabolites (d-ROMs), (B) biological antioxidant potential (BAP) and (C) oxidative stress index [(d-ROMs/BAP) × 100] in live yeast (LY) supplemented and control cows during the study period. *Values are significantly different between supplemented and control cows within a sample period at P < 0.05.

Fig. 4. (A) Mean serum concentrations (± SE) of retinol, (B) α-tocopherol and (C) β-carotene in live yeast (LY) supplemented and control cows during the study period. *, **Values are significantly different between supplemented and control cows within a sample period at P < 0.05 and P < 0.01, respectively.

Fig. 5. (A) Mean concentrations (± SE) of serum glucose, (B) blood urea nitrogen (BUN) and (C) serum total cholesterol (T-Cho) in live yeast (LY) supplemented and control cows during the study period. **Values are significantly different between supplemented and control cows within a sample period at P < 0.01.
Table 1. Changes in rectal temperature, respiration rate, body condition score and hematological values after live yeast (LY) supplementation

| Parameter      | Treatment     | Weeks relative to treatment |
|----------------|---------------|-----------------------------|
|                |               | W0  | W1  | W2  | W4  | W6  | W8  |
| Rectal temperature (°C) | LY            | 38.8 ± 0.1 | 38.8 ± 0.1 | 38.8 ± 0.1 | 39.2 ± 0.1 | 38.7 ± 0.1 | 38.7 ± 0.1 |
|                 | Control       | 38.9 ± 0.1 | 38.9 ± 0.2 | 38.8 ± 0.1 | 39.0 ± 0.2 | 38.7 ± 0.1 | 38.8 ± 0.1 |
| Respiration rate (breaths/min) | LY            | 43.0 ± 3.9 | 40.1 ± 2.9 | 60.4 ± 6.7 | 63.0 ± 5.8 | 39.5 ± 3.0 | 45.5 ± 3.9 |
|                 | Control       | 47.3 ± 3.5 | 39.4 ± 2.2 | 57.4 ± 3.1 | 61.1 ± 3.6 | 38.3 ± 1.3 | 40.5 ± 3.2 |
| Body condition score (1 to 5) | LY            | 3.00 ± 0.07 | 3.00 ± 0.07 | 2.97 ± 0.07 | 3.03 ± 0.07 | 3.19 ± 0.09 | 3.13 ± 0.08 |
|                 | Control       | 3.03 ± 0.07 | 3.03 ± 0.07 | 2.97 ± 0.06 | 3.03 ± 0.06 | 3.06 ± 0.06 | 3.00 ± 0.07 |
| White blood cell (×10^2/µl) | LY            | 83.6 ± 5.1 | 79.9 ± 5.1 | 81.4 ± 5.5 | 84.1 ± 5.0 | 75.3 ± 4.4 | 82.3 ± 7.5 |
|                 | Control       | 88.9 ± 5.7 | 85.9 ± 4.9 | 84.9 ± 5.2 | 77.9 ± 4.1 | 81.9 ± 6.4 | 89.9 ± 8.1 |
| Red blood cell (×10^4/µl) | LY            | 661.4 ± 30.1 | 603.3 ± 22.1 | 606.3 ± 20.5 | 570.3 ± 44.3 | 609.9 ± 21.1 | 609.0 ± 18.4 |
|                 | Control       | 625.1 ± 34.5 | 576.3 ± 16.0 | 574.5 ± 15.4 | 568.6 ± 20.2 | 594.3 ± 11.1 | 592.1 ± 15.7 |
| Hemoglobin (g/dl) | LY            | 10.5 ± 0.4 | 9.7 ± 0.3 | 9.6 ± 0.3 | 9.7 ± 0.2 | 9.9 ± 0.2 | 9.9 ± 0.2 |
|                 | Control       | 10.5 ± 0.6 | 9.7 ± 0.2 | 9.7 ± 0.2 | 9.5 ± 0.3 | 10.0 ± 0.2 | 9.9 ± 0.3 |
| Hematocrit (%) | LY            | 30.2 ± 1.3 | 27.7 ± 0.9 | 27.7 ± 0.7 | 28.0 ± 0.7 | 28.3 ± 0.7 | 28.3 ± 0.7 |
|                 | Control       | 30.2 ± 1.7 | 27.7 ± 0.7 | 27.5 ± 0.7 | 27.5 ± 1.0 | 28.9 ± 0.5 | 28.6 ± 0.7 |
| Platelet (×10^4/µl) | LY            | 33.1 ± 3.8 | 32.7 ± 2.4 | 37.5 ± 2.0 | 35.4 ± 3.6 | 35.4 ± 4.0 | 33.1 ± 2.3 |
|                 | Control       | 37.1 ± 2.7 | 33.0 ± 3.1 | 35.8 ± 1.9 | 36.5 ± 3.3 | 34.9 ± 3.6 | 33.2 ± 5.2 |

RT, rectal temperature; RR, respiration rate; BCS, body condition score; WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; PLT, platelet.

Data are expressed as mean ± SE.
Table 2. Changes in lactation performances after live yeast (LY) supplementation

| Parameter                  | Treatment | Days relatives to treatment |
|----------------------------|-----------|----------------------------|
|                            |           | Day 0       | Day 32       | Day 63       |
| Milk yield (kg/d)          | LY        | 28.2 ± 2.4  | 25.5 ± 2.6  | 23.5 ± 2.7  |
|                            | Control   | 27.6 ± 1.1  | 25.1 ± 0.9  | 22.2 ± 1.6  |
| Reduction rate of milk     | LY        | –            | 10.0 ± 2.8  | 17.6 ± 4.9  |
| yield (%)                  | Control   | –            | 9.0 ± 1.8   | 20.0 ± 4.3  |
| Milk fat (%)               | LY        | 3.89 ± 0.16  | 3.98 ± 0.20 | 4.13 ± 2.74 |
|                            | Control   | 3.50 ± 0.15  | 3.75 ± 0.13 | 3.87 ± 0.14 |
| Milk protein (%)           | LY        | 3.36 ± 0.16  | 3.45 ± 0.15 | 3.77 ± 0.12 |
|                            | Control   | 3.35 ± 0.09  | 3.48 ± 0.08 | 3.68 ± 0.09 |
| Solids-not-fat (%)         | LY        | 8.72 ± 0.15  | 8.84 ± 0.14 | 9.18 ± 0.10 |
|                            | Control   | 8.73 ± 0.12  | 8.90 ± 0.09 | 9.13 ± 0.09 |

Data are expressed as mean ± SE.
Fig. 1
Fig. 3

Serum d-ROMs (U. CARR)

Weeks relative to treatment

Supplemented  Control
Fig. 3

B 3500
3000
2500
2000
1500
1000
500
0

Serum BAP (µmol/l)

Supplemented
Control

W 0  W 1  W 2  W 4  W 6  W 8

Weeks relative to treatment
Fig. 3

Oxidative stress index [(d-ROMs/BAP) × 100]

- Supplemented
- Control

Weeks relative to treatment
Fig. 4
Fig. 4

Weeks relative to treatment

α-Tocopherol (μg/dL)

Supplemented
Control
Fig. 4

β-Carotene (μg/dl)

Weeks relative to treatment

Supplemented vs Control
**Fig. 5**

![Bar chart showing glucose levels over weeks relative to treatment.](image-url)
Fig. 5

BUN (mg/d\text{L})

Weeks relative to treatment

Supplemented
Control

W 0  W 1  W 2  W 4  W 6  W 8

Fig. 5
Fig. 5

A graph showing the total cholesterol levels (mg/dL) over 8 weeks relative to treatment. The x-axis represents weeks (W 0 to W 8) and the y-axis represents total cholesterol levels (mg/dL) ranging from 0 to 240. Two lines are plotted: one for Supplemented and one for Control groups. The Supplemented group shows a slight decrease in cholesterol levels over time, while the Control group shows a slight increase. The graph includes error bars indicating variability.