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The effect of spirulina supplementation on calf growth, biochemical status and oxidative stress: A meta-analysis

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Abstract

This meta-analysis aims to evaluate the effects of Spirulina platensis supplementation on calf growth performance, biochemical status, and oxidative stress. A comprehensive literature search totaling 514 studies was conducted in May and June 2024 using Scopus and Google Scholar, employing keywords: Spirulina, calf, growth performance, biochemical and antioxidant. Original research articles that reported mean, standard deviation, and sample size data on calves supplemented with spirulina were included in the analysis. The selected papers are followed by a meta-analysis process using Open MEE software. The effect size was calculated as standardized mean differences between treatments by comparing spirulina supplementation to the control group. A systematic review study shows that the duration of Spirulina supplementation ranges between 40 and 180 days and focused on calves with body weights ranging from 32-160 kg. The meta-analysis results showed that Spirulina supplementation had a significant ($P < 0.05$) effect on body weight, average daily gain, catalase, glutathione, malondialdehyde, superoxide dismutase, total antioxidant capacity, erythrocytes, hemoglobin, and uric acid, compared to the control group. These findings suggest that the use of spirulina can enhance antioxidant potential and improve overall health and metabolic function in supplemented calves. In conclusion, Spirulina supplementation demonstrates a positive impact on improving calves' growth performance, biochemical status, and antioxidant status. Based on these various studies, we recommend considering the inclusion of spirulina as a potential supplement in calf feeding programs.

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Introduction

The global meat industry continues to grow due to changing lifestyles, preferences and cultural factors (1) such as rising incomes in developing countries. Meat is a significant source of nutrition, providing 11% of global energy and 21% of protein, with ruminant meat contributing 23% (2). Given its contribution to global nutrition, it is crucial to develop the livestock industry to provide quality and healthy meat while prioritizing animal welfare. Livestock experience stress, which negatively impacts their immune response and performance. For example, despite being relatively heat tolerant, calves still suffer from heat stress to a certain degree, which can be a significant stressor (3). Stress in livestock occurs when environmental or management factors such as thirst, hunger, injury, or extreme temperatures disrupt their normal physiological state. Animals respond to stress by adapting their physiology to cope, but chronic stress can negatively impact their immune response and performance (4). Even when calves experience stress during weaning, it can affect their immunity during the transition from individual pens to group pens (5). Therefore, there is an increase in demand for antioxidants to reduce the adverse effects of free radicals on the immune system.

Utilizing natural antioxidants like spirulina as a feed supplement offers a safe and environmentally friendly approach for farmers, aligning with the principles of the green economy. Research suggests that spirulina's antioxidant properties can support the immune system and potentially mitigate stress in livestock (6). Studies have shown that Spirulina supplementation may benefit calves by mitigating the negative effects of stress on growth (7) and improving antioxidant capacity and weight gain. Additionally, research indicates positive effects on rumen health and feed consumption, especially when fed with low-quality forages (8). Spirulina is a species of filamentous cyanobacteria that contains chlorophyll, carotenoids, phycocyanin, and phenolic compounds, which can be used as natural antioxidants (9). Spirulina activates cellular antioxidant enzymes, inhibits lipid peroxidation and DNA damage, scavenges free radicals, and increases superoxide dismutase (SOD) and catalase activity. Spirulina stimulates antibody production and modulates the expression of cytokine-encoding genes, inducing immunomodulatory and anti-inflammatory responses. Phycocyanin and beta-carotene are important molecules that support these processes (10). C-phycocyanin, a very dominant protein pigment in spirulina, possesses numerous biological activities, including antioxidant and anti-inflammatory properties. It can trigger a more significant increase in blood SOD activity than vitamin E (11). These findings highlight the promising potential of spirulina as a cost-effective and sustainable resource for enhancing livestock production (12). A meta-analysis approach could be particularly valuable in addressing this knowledge gap. Analyzing data from multiple studies can provide a more comprehensive understanding of spirulina's overall effect on calf health, growth, and production efficiency, while increasing statistical power and summarizing findings for a clearer picture (13,14).

This study hypothesizes that feed supplementation using spirulina positively affects the performance of calves, specifically in terms of growth performance, biochemical status, and oxidative stress. This supplementation has the potential to reduce stress levels and improve overall health and growth in livestock. The aim of this study is to evaluate the benefits of Spirulina supplementation on growth performance, biochemical status, and oxidative stress in calves through a meta-analysis approach.

Materials and methods

Ethical approval

This research is an article review in the form of a meta-analysis, so it does not use ethical approval.

Study period and location

This review was conducted between May and June 2024. Studies published between 2014 and 2024 were considered.

Meta-analysis

A systematic and comprehensive search for papers was carried out by adopting the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) protocol by Page *et al.* (15), as presented in figure 1. Meta-analysis was used in this study to address the following research questions: how does Spirulina supplementation affect calves' growth performance (body weight and average daily gain)? How does Spirulina supplementation affect the antioxidant status of calves (catalase (CAT), glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), and total antioxidant capacity (TAC)). How does Spirulina supplementation affect the haematological and biochemical status of calves (erythrocytes, leukocytes, hemoglobin, albumin, glucose, low-density lipoprotein (LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine, urea, and uric acid). A comprehensive search for relevant studies was conducted using the PICO framework (16). The details of the PICO framework are as follows: population (calves, cattle), intervention (Spirulina), comparison (control), and outcome (growth performance, antioxidant, and biochemical parameters). The databases used were Google Scholar and Scopus. Based on this concept, the keywords used for searching meta-analysis papers were calves, cattle, spirulina, growth performance, antioxidant, and biochemical.

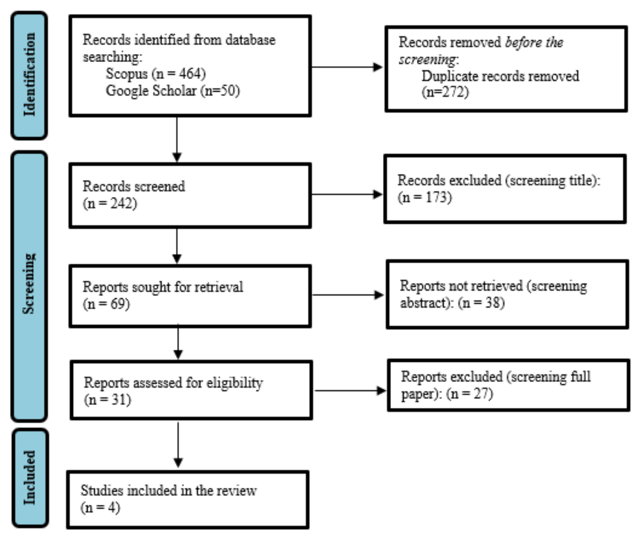

Figure 1: A PRISMA diagram that simplifies the paper selection strategy for the meta-analysis process.

The following inclusion criteria were adopted from Trevi *et al.* (17) and adjusted to the research objectives: (1) is an original study with a population of calves; (2) written in English; (3) Spirulina as a feed supplement; (4) presents data on the average value of the variable, standard deviation, and number of samples. A flowchart (Figure 1) depicts the paper selection procedure for this meta-analysis. The initial search through Scopus and Google Scholar databases yielded 514 potential papers. After removing duplicates (272), 242 papers remained. Title screening excluded 173 papers, leaving 69 for abstract screening. Based on the availability of population data, sample size, and suitability of variables, 38 papers were excluded during the abstract screening. Further screening was conducted to identify papers with data on mean values, standard deviations, and sample size for the variables of interest. This resulted in the exclusion of 27 additional papers. Four papers published between 2018 and 2023 were deemed suitable for inclusion in the meta-analysis.

Data extraction

Meta-analysis data was obtained from selected papers, followed by tabulation and meta-analysis through data collection as follows: (1) first author and year of publication; (2) the average value of the variables from the treatment group (Xe) and the control group (Xc); (3) standard deviation of treatment (Se) and control (Sc) groups; (4) number of animals in the treatment (Ne) and control (Nc) groups; (5) dosage of Spirula use; and (6) country of research location.

Data analysis

Meta-analysis used OpenMEE software (18). The effect size was calculated as standardized mean differences (SMD), Hedges'd, between the spirulina and control diets. After data inspection, we used a random effect model to obtain the overall effect because a common underlying effect (fixed effect model) cannot be assumed. The mathematical models applied are as follows:

$$Y_i = \mu + \tau_i + \varepsilon_i \quad (1)$$

$$Q = \sum(w_i\, ES_i^2) - \frac{(\sum(w_i ES_i))^2}{\sum w_i} \quad (2)$$

$$\tau^2 = \frac{Q-df}{C} \quad (3)$$

$$I^2 = \frac{(Q-(k-1))}{Q} \times 100 \quad (4)$$

Where yi is the diversity of the effect size, μ is the mean true effect, τi is the diversity of the true effect size, and εi is the sampling error (19). Three measures of between-study heterogeneity: Cochran's Q, an I^2 index that varies from <25% to >75%, and tau-squared (τ^2), which represents variation between studies using the DerSimonian and Laird method. Where wi is the weighting of each study, ES is the effect size value, k is the number of studies analyzed, df is the degree of freedom, and C is the estimated value. These studies originated from three countries (Malaysia, Egypt, Russia) and focused on calves aged 1-12 months with body weights ranging from 32-160 kg (Table 1).

Table 1: Description of the studies in the meta-analysis database

References	Duration	Dose	Breed	Country
Ilona *et al.* (20)	60 days	10-15 mg DM/kg BW	Holstein	Russia
Ghattas *et al.* (21)	45 days	6 g/h/d	Holstein	Egypt
Shamsudin *et al.* (22)	40 days	2-4 g/10 kg BW/h/d	Kelantan	Malaysia
Mohamed *et al.* (23)	180 days	0.5 g/kg feed NRC/h/d	Holstein	Egypt

DM=dry matter, BW=body weight, NRC= National Research Council, h=head, d=day.

Results

Growth performance and antioxidant status

The meta-analysis of growth performance variables in calves (Table 2), based on the cumulative effect size values, shows that spirulina has a real effect on body weight and average daily gain. The meta-analysis showed that body weight and average daily gain values increased following Spirulina supplementation, with a difference between treatment and control scores of 0.85 and 14.43, respectively.

The results of the meta-analysis of antioxidant status variables in calves (Table 2), based on cumulative effect size values, suggest that Spirulina supplementation has a real effect on multiple antioxidant variables. These include catalase, glutathione, superoxide dismutase, and total antioxidant capacity, which all showed statistically significant increases ($P<0.05$) after Spirulina supplementation compared to the control group. The effect sizes were interpreted as moderate for catalase (7.71), glutathione (1.24), superoxide dismutase (3.68) and large for total antioxidant capacity (4.85). Malondialdehyde, a marker of oxidative stress, showed a statistically significant decrease (-14.02, $P<0.05$) after Spirulina supplementation.

Hematological and biochemical analysis

The results of the meta-analysis of haematological and biochemical analysis variables in calves (Table 3), based on the cumulative effect size value, show that Spirulina supplementation has a real effect on several blood parameters. These include erythrocytes (red blood cells), haemoglobin (an iron-rich protein in red blood cells that carries oxygen), and uric acid (a waste product). Haemoglobin and uric acid levels increased after supplementation with spirulina compared to the control group.

Table 2: Growth performance and antioxidant status of calves supplemented with spirulina

Item	Ns	X c	Xe	Unit	Model results			Heterogeneity			
					Estimate	SE	p-Value	tau^2	Q	P Value	I^2
BW	6	99.2[b]	101.3[a]	kg	0.85	0.37	0.021	0.44	11.05	0.050	54.73
ADG	4	0.60[b]	0.64[a]	kg	14.48	2.78	<0.001	24.87	17.85	<0.001	83.19
CAT	3	40.8[b]	58.1[a]	IU/l	7.71	2.14	<0.001	11.84	16.21	<0.001	87.66
GSH	3	55.4[b]	62.4[a]	n/ml	1.24	0.48	0.010	0.45	5.58	0.061	64.18
MDA	3	13.8[a]	4.20[b]	mmol/l	-14.02	1.52	<0.001	1.72	2.64	0.267	24.30
SOD	3	13.3[b]	17.3[a]	n/ml	3.68	0.99	<0.001	2.36	10.46	0.005	80.87
TAC	3	432[b]	533[a]	n/ml	4.85	1.94	0.012	10.19	30.73	<0.001	93.49

Ns=total sample, Xc = mean value of the variable from the control group, Xe= mean value of the variable from the treatment group, BW=bodyweight, ADG=average daily gain, CAT=catalase, GSH=glutathione, MDA=malondialdehyde, SOD=superoxide dismutase, TAC=total antioxidant capacity, SE=standard of errors. [a,b]= Different letters in the diagram on the same row indicate significant differences based on meta-analysis subgroups.

Table 3: Haematological and biochemical analysis of calves supplemented with *Spirulina platensis*

Item	Ns	X c	Xe	Unit	Model results			Heterogeneity			
					Estimate	SE	p-Value	tau^2	Q	P Value	I^2
Erythrocytes	5	6.71[b]	6.99[a]	10^{12}/l	-0.45	0.23	0.047	0.04	4.75	0.314	16.77
Leucocytes	5	9.55	8.72	10^9/l	-0.27	0.40	0.496	0.58	14.08	0.007	71.59
Haemoglobin	5	87.8[b]	91.9[a]	g/l	0.96	0.28	<0.001	0.14	6.40	0.171	37.49
Albumin	14	2.33	2.39	g/l	0.09	0.25	0.728	0.47	29.48	0.006	55.90
Glucose	7	47.7	45.0	mg/l	-0.64	0.48	0.182	1.37	40.11	<0.001	85.04
LDL	10	1.18	1.2	mol/l	0.22	0.30	0.461	0.40	16.19	0.063	44.4
AST	12	66.6	69.4	IU/l	-0.39	0.36	0.266	1.07	37.68	<0.001	70.81
ALT	12	48.8	52.5	IU/l	-0.40	0.55	0.460	3.05	74.91	<0.001	85.32
BUN	9	8.68	9.53	mg/dl	-0.08	0.48	0.874	1.41	26.37	<0.001	69.66
Creatinine	12	73.5	81.4	mol/l	-0.11	0.41	0.800	1.52	47.16	<0.001	76.67
Urea	12	10.2	9.38	mol/l	-0.55	0.39	0.167	1.36	43.46	<0.001	74.69
Uric acid	9	169[b]	209[a]	mol/l	0.95	0.35	0.006	0.46	14.02	0.081	42.92

Ns=total sample, Xc = mean value of the variable from the control group, Xe= mean value of the variable from the treatment group, LDL= low-density lipoprotein, AST= aspartate aminotransferase, ALT= alanine aminotransferase, BUN= blood urea nitrogen, SE=standard of errors. [a,b]= Different letters in the diagram on the same row indicate significant differences based on meta-analysis subgroups.

Discussions

Selected papers from the results of the meta-analysis in this study using *Spirulina platensis*. *Spirulina platensis* contains 11 mg/g of chlorophyll and 7.2 mg/g of carotenoids (24). Gaafar *et al.* (25) reported that spirulina can trigger improvements in cow body weight by a percentage of up to 6.58%, allegedly due to increasing Spirulina feed intake and improving energy balance. Berah *et al.* (7) reported that the daily body weight gain of calves given 3% spirulina had a greater ADG than the control. *Spirulina platensis* has a more important effect in supplying protein and enhancing the intake of animals on low crude protein, which is what most calves are faced with. Riad *et al.* (26) reported an increase in average daily gain and total plasma protein after *spirulina platensis* supplementation of 1-2 g/head/day in Friesian calves. Spirulina supplementation increases the benefits of bacteria in the rumen and rumen papillae, which are important for nutrient absorption (27). This is useful for increasing feed efficiency, as well as increasing average daily gain. Berah *et al.* (7) reported that calves given spirulina increased SOD and TAC concentrations in the group given 1% of the feed. An increase in GSH indicates increased oxidative defence of animal tissue. The antioxidant effect of spirulina is related to several active ingredients, such as alpha-tocopherol and beta-carotene, which work individually or in synergy against free radicals (28). Spirulina improves various symptoms caused by oxidative stress in the body, such as repairing nerve damage, inhibiting inflammation, increasing immunity, reducing toxicity, improving organ function, and alleviating cardiovascular disease conditions (29).

Blood tests are indicators of health in livestock. Positive results for several blood haematologic parameters can be attributed to spirulina's crude protein content, folic acid, vitamins, and other macro and micro elements (21,30).

Based on blood biochemical observations, spirulina does not cause a significant effect on blood liver injury biomarkers (AST and ALT), which shows that spirulina can protect liver function by containing the blue pigment phycocyanin which reduces liver toxicity caused by free radicals (31). Shamsudin *et al.* (22) reported that Spirulina platensis increased HDL levels, reduced cholesterol and stabilized blood BUN, LDL, and ALT levels in cows without showing adverse effects on kidney and liver function. These findings suggest that Spirulina platensis can be safely incorporated into calf diets without compromising health or productivity.

Conclusion

In conclusion, *Spirulina platensis* supplementation demonstrated the potential to enhance calf growth performance, improve biochemical status, and mitigate oxidative stress. These findings suggest that *Spirulina platensis* could be a valuable strategy to enhance calf health and productivity.

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Conflict of interest

There is no conflict of interest.

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تأثير مكملات السبيرولينا على نمو العجل والحالة البيوكيميائية والإجهاد التأكسدي: تحليل تلوي

فريدانسياه فردوس، تاندا ساهات بانجايتان، يودي أديناتا، نور هديا كريشنا، بايو أندري أتموكو، ريتنو ويدياواتي، ومالك مكمور

مركز أبحاث الثروة الحيوانية، الوكالة الوطنية للبحث والابتكار، مركز سيبينونج للعلوم، بوجور، إندونيسيا

الخلاصة

يهدف هذا التحليل التلوي إلى تقييم آثار مكملات سبيرولينا بلاتنسيس على أداء نمو العجل، والحالة الكيميائية الحيوية، والإجهاد التأكسدي. تم إجراء بحث شامل في المصادر بلغ مجموعها ٥١٤ دراسة في أيار وحزيران ٢٠٢٤ في موقعي Scopus و Google Scholar، باستخدام كلمات رئيسية: سبيرولينا، العجل، أداء النمو، الكيمياء الحيوية ومضادات الأكسدة. تم تضمين المقالات البحثية الأصلية التي أبلغت عن بيانات المتوسط والانحراف المعياري وحجم العينة على العجول المكملة بالسبيرولينا في التحليل. أجري على المصادر المختارة عملية تحليل تلوي باستخدام برنامج Open MEE. تم حساب حجم الأثر كفروق متوسطية معيارية بين العلاجات بمقارنة التكملة بالسبيرولينا بمجموعة السيطرة. أظهرت دراسة مراجعة منهجية أن مدة مكملات سبيرولينا تتراوح بين ٤٠ و ١٨٠ يوما وتركز على العجول التي تتراوح أوزانها من ٣٢-١٦٠ كجم. أظهرت نتائج التحليل التلوي أن مكملات سبيرولينا كان لها تأثير معتد به ($P<0.05$) على وزن الجسم، ومتوسط الكسب اليومي، والكاتالاز، والجلوتاثيون، ومالوند ديالديهايد، وسوبر أكسيد ديسموتاز، والقدرة الإجمالية المضادة للأكسدة، وكريات الدم الحمراء، والهيموجلوبين، وحمض اليوريك، مقارنة بمجموعة السيطرة. تشير هذه النتائج إلى أن استخدام السبيرولينا يمكن أن يعزز إمكانات مضادات الأكسدة ويحسن الصحة العامة ووظيفة التمثيل الغذائي في العجول المكملة. في الختام، تظهر مكملات سبيرولينا تأثيرا إيجابيا على تحسين أداء نمو العجول والحالة الكيميائية الحيوية وحالة مضادات الأكسدة. بناء على هذه الدراسات المختلفة، نوصي بالنظر في إدراج السبيرولينا كمكمل محتمل في برامج تغذية العجول.