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Professional paper

THE EFFECT OF SINAPIS ESSENTIAL OIL ON CHICKEN EMBRYO DEVELOPMENT: A COMPARATIVE EXPERIMENTAL STUDY

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Abstract

This study investigates the effect of Sinapis essential oil (*Brassica juncea*) on the embryonic development of chickens. Known for its antimicrobial, antioxidant, and growth-stimulating properties, Sinapis essential oil was selected to explore its potential influence during embryogenesis. Thirty fertilized chicken eggs were divided into two equal groups: 15 eggs in the control group (untreated), and 15 eggs in the experimental group, which received 0.05 ml (1.5%)of Sinapis essential oil injected into the air cell on day 1 of incubation.
Embryonic development was monitored on days 7, 14, and 21. Measurements included egg weight (recorded weekly), embryo weight, length, width, and eye size, along with visual assessment of organ differentiation. Results showed embryos in the treated group had superior growth: embryo weight increased by 1–1.5 g, average length reached 3.0 cm (vs. 2.5 cm), width 2.4 cm (vs. 1.8 cm), and eye diameter 0.7 mm (vs. 0.5 mm) compared to controls. Organs were visibly more defined in the treated group.
Data analysis using SPSS Two-way ANOVA, confirming statistically significant differences ($p < 0.05$) between groups. These findings suggest that Sinapis essential oil may positively influence embryo development in avian species.

Keywords: Sinapis essential oil, embryonic development, incubation, chicken embryo, SPSS, t-test, ANOVA, organ differentiation

1. Introduction

The embryonic development of the chicken is a classical and widely utilized model in biological research due to its accessibility, clearly defined developmental stages, and the possibility of experimental intervention without the need for invasive procedures in a living organism. Chicken eggs provide a closed and controllable system, allowing researchers to assess the impact of various substances on embryonic development from the first day of incubation until hatching.
In recent years, there has been a growing interest in the use of natural substances with positive biological effects, both in veterinary sciences and biotechnology. Mustard oil (*Brassica juncea*), extracted from the seeds of the Brassicaceae plant, is known for its rich content of bioactive compounds such as isothiocyanates, natural antioxidants, and essential fatty acids. These compounds have demonstrated antibacterial, anti-inflammatory properties, and the potential to enhance cellular metabolism.
Despite its traditional use in folk medicine and as an ingredient in certain foods, the application of mustard oil in developmental biology remains limited and requires further controlled studies to evaluate its potential effects on embryonic development. Against this background, the aim of this study was to assess the effect of mustard oil on the embryonic development of chickens through direct in ovo application.
In this experiment, 30 chicken eggs were used, divided into two groups: a control group with no treatment, and an experimental group in which 0.05 ml of mustard oil (1.5%) was injected into the albumen cavity of the egg. All eggs were incubated under standard embryonic development conditions for a period of 21 days. The eggs were weighed before treatment and

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every 7 days during incubation. Embryos were analyzed on days 7, 14, and 21 through morphometric measurements such as body weight, length, body width, and eye size, as well as through visual assessment of internal organ development.
This study provides an initial contribution to evaluating the use of mustard oil (*Brassica juncea* oil) as a potential agent for enhancing embryonic development and opens avenues for further research into its effects in various biological systems and in the natural growth promotion of poultry.

2. Materials and Methods

The study was conducted on a total of 30 fertilized chicken eggs, divided equally into two groups: a control group (n=15) and an experimental group (n=15). The experimental group was treated with 0.05 ml of essential mustard oil (*Brassica juncea* oil) at a concentration of 1.5%, injected into the air cell of the egg on the first day of incubation. The control group remained untreated and served as a baseline for comparison.

Incubation conditions:

All eggs were incubated under standard conditions at a temperature of 37.8°C and relative humidity of 60–65%. Automatic rotation of the eggs was performed every 6–8 hours. The incubation period lasted for 21 days.

Objective of the Experiment:

The aim of this experiment was to evaluate the impact of mustard oil (*Brassica juncea*) on the embryonic development of chickens by comparing treated and untreated eggs.

Measurements and Observations:

Egg weight was recorded prior to treatment and every 7 days throughout the incubation period. Embryonic development was monitored on days 7, 14, and 21. On these days, the following parameters were measured: embryo weight, embryo length, eye diameter, as well as a visual assessment of general embryonic development.

3. Results and Discussion

Table 1. *Effect of Time and Treatment Interaction on the Weight of Chicken Eggs*

Tests of Between-Subjects Effects

Dependent Variable: peshaV

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4777.033[a]	7	682.433	37.340	.000
Intercept	318064.033	1	318064.033	17403.191	.000
dita	4402.500	3	1467.500	80.296	.000
grupi	374.533	1	374.533	20.493	.000
dita * grupi	.000	3	.000	.000	1.000
Error	2046.933	112	18.276		
Total	324888.000	120			
Corrected Total	6823.967	119			

a. R Squared = .700 (Adjusted R Squared = .681)

The effect of time (day) was statistically significant (p = .000), indicating that the weight of the eggs changed significantly across the incubation days (7, 14, and 21).
The effect of the group (treatment vs. control) was also statistically significant (p = .000), suggesting that mustard oil treatment had a significant impact on egg weight.
However, the interaction effect between day and group was not statistically significant (p =

1.000), meaning that the effect of mustard oil remained consistent throughout the incubation period and did not vary across different days.

Table 2. *Post Hoc Tukey HSD Test*

Multiple Comparisons

Dependent Variable: peshaV ...

		Mean Difference (I-			95% Confidence Interval	
1	7	3.0000*	1.10382	.038	.1212	5.8788
	14	8.0000*	1.10382	.000	5.1212	10.8788
	21	16.0000*	1.10382	.000	13.1212	18.8788
7	1	-3.0000*	1.10382	.038	-5.8788	-.1212
	14	5.0000*	1.10382	.000	2.1212	7.8788
	21	13.0000*	1.10382	.000	10.1212	15.8788
14	1	-8.0000*	1.10382	.000	-10.8788	-5.1212
	7	-5.0000*	1.10382	.000	-7.8788	-2.1212
	21	8.0000*	1.10382	.000	5.1212	10.8788
21	1	-16.0000*	1.10382	.000	-18.8788	-13.1212
	7	-13.0000*	1.10382	.000	-15.8788	-10.1212

Based on observed means.
The error term is Mean Square(Error) = 18.276.

*. The mean difference is significant at the 0.05 level.

The results of the Post Hoc Tukey HSD test revealed that egg weight decreased significantly from day 1 to day 21 of incubation.
All differences between days were statistically significant ($p < 0.05$).
Specifically, the average weight decreased from 58.2 g on day 1 to 42.2 g on day 21, with a progressive loss of weight at each stage.
This change may be attributed to physiological processes occurring during embryonic development, including water loss and the utilization of nutritional reserves.

Embryo Weight

Table 3. Interaction effect of group and day on embryo weight on days 7, 14, and 21

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	4303.567[a]	5	860.713	1043.289	.000	.995
Intercept	8944.133	1	8944.133	10841.374	.000	.998
groups	45.633	1	45.633	55.313	.000	.697
day	4246.667	2	2123.333	2573.737	.000	.995
group * day	11.267	2	5.633	6.828	.004	.363
Error	19.800	24	.825			
Total	13267.500	30				
Corrected Total	4323.367	29				

a. R Squared = .995 (Adjusted R Squared = .994)

$R^2 = .995$, indicating that 99.5% of the variance in the weight of the treated eggs is explained by these factors.

- There is a statistically significant effect of day on embryo weight ($p < .001$), with a clear progressive increase in weight from day 7 to day 21.
- There is also a significant effect of group (control vs. treatment) on embryo weight ($p < .001$), with the group treated with Brassica juncea oil showing higher weight values at all developmental stages.

• The interaction between group and day was statistically significant (p = .004), suggesting that the effect of Brassica juncea oil changes over time and becomes more pronounced as incubation progresses.

Table 4. Post Hoc Tukey HSD test.

Multiple Comparisons
Dependent Variable: Embryo Weight
Tukey HSD

(I) day	(J) day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
day7	day14	-12.0000*	.40620	.000	-13.0144	-10.9856
	day21	-29.0000*	.40620	.000	-30.0144	-27.9856
day14	day7	12.0000*	.40620	.000	10.9856	13.0144
	day21	-17.0000*	.40620	.000	-18.0144	-15.9856
day21	day 7	29.0000*	.40620	.000	27.9856	30.0144
	day 14	17.0000*	.40620	.000	15.9856	18.0144

Based on observed means.
The error term is Mean Square(Error) = .825.
*. The mean difference is significant at the .05 level.

From the Tukey HSD table, it is observed that the weight on days 21 and 14 is significantly higher compared to day 7 ($p = 0.000$). Additionally, the experimental egg weights on day 21 are significantly greater than those on days 14 and 7 ($p = 0.000$). All differences between days are statistically significant → ***Weight changes significantly from day to day.***
Post hoc comparisons (Tukey HSD) confirmed that the weight differences between days were statistically significant for all pairwise comparisons (day 7 vs. 14, 14 vs. 21, and 7 vs. 21), both for the control and the treated groups.

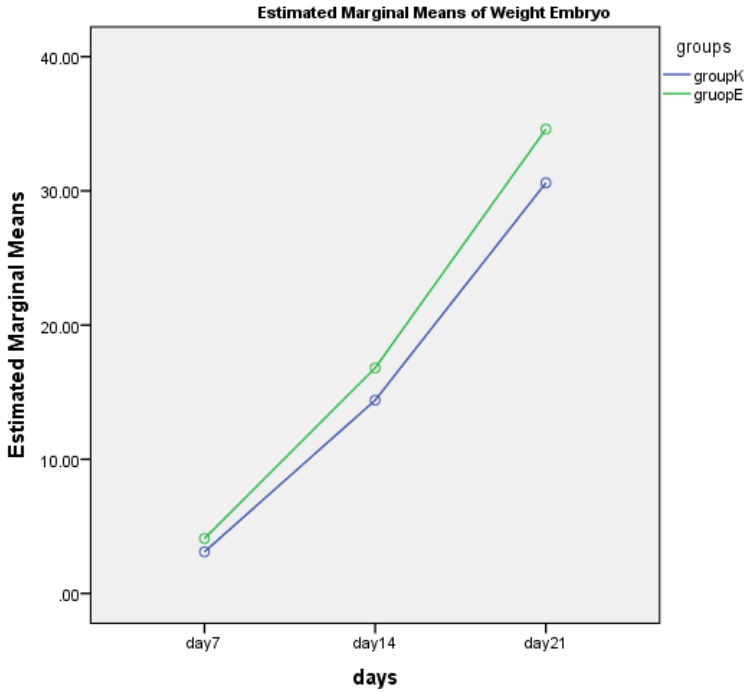


Figure 1. Changes in egg weight over days 7, 14, and 21 across groups.

- The graph illustrates the change in experimental egg weight as a function of incubation day, comparing two different groups.
- Both groups show a steady increase in weight over time, but the group represented by the green color (Group 2 – treated eggs) consistently exhibits higher weights at each time point compared to the other group (Group 1 – control).
- The plot of estimated marginal means clearly demonstrates an increasing trend in embryo weight, with the treated group always showing greater weight than the control group at all three developmental stages.

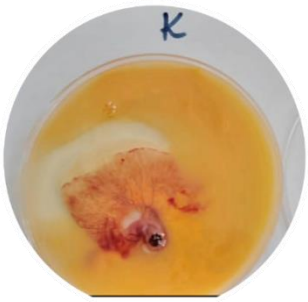
Table 5. Embryo length on day 7 (cm)

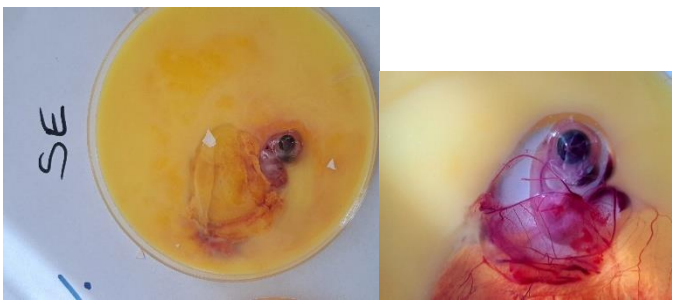
K1	K2	K3	k4	K5	S1	S2	S3	S4	S5
1.3	1.5	1.1	1.4	1.6	1.9	1.7	1.8	1.6	1.7

Table 6. Eye size (cm)

Group/day	7	14	21
K1	0.5	0.6	0.7
K2	0.5	0.6	0.7
K3	0.5	0.6	0.7
k4	0.5	0.6	0.7
K5	0.5	0.6	0.7
S1	0.8	0.8	0.8
S2	0.8	0.8	0.8
S3	0.8	0.8	0.8
S4	0.8	0.8	0.8
S5	0.8	0.8	0.8

On day 8 of incubation, noticeable developments were observed in the eggs treated with mustard oil (*Brassica juncea*). At this stage, embryos in the treated eggs exhibited more developed organs compared to the control group. Key organs such as the heart, brain, and nervous system were significantly more developed. Embryos in the treated group showed a faster heart rate and more pronounced development of nervous structures, suggesting an acceleration of the developmental process compared to the control eggs.
The degree of organ differentiation was visually assessed and documented through photographs for comparison between groups.





Photographs from day 7 of the control and treated groups

On day 7 of incubation, noticeable developments were observed in the eggs treated with mustard oil (*Brassica juncea*). At this stage, embryos in the treated eggs exhibited more developed organs compared to the control group. Key organs such as the heart, brain, and nervous system were markedly more developed. Embryos in the treated group also showed a faster heart rate and clearer development of nervous structures, suggesting an accelerated developmental process compared to embryos in the control group.

Control **Treated with**

***Brassica juncea* oil**

Photographs from day 14 of the control and treated groups

Control **Treated**

Photos from day 21

On days 14 and 21, embryonic development continued to be faster and more pronounced in the treated group. The treated eggs were also more likely to hatch chicks compared to the control group. This suggests that mustard oil may have a positive effect on accelerating embryonic development.

4. Conclusion

The results of this experiment indicate that mustard oil has a significant impact on the embryonic development of chicks. The faster and more pronounced development of embryos in eggs treated with mustard oil may be related to the fatty acid content of the oil, which could enhance metabolism and stimulate cell growth. Despite these positive findings, further studies are necessary to better understand the biological mechanisms underlying this effect and to evaluate the safety and efficacy of the treatment under different conditions. Eggs treated with mustard oil showed accelerated development of key organs and earlier hatching of chicks. These results suggest a potential application of mustard oil in improving embryonic development in

the poultry industry; however, further research is needed to assess the long-term impacts of this treatment.

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