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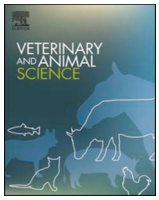

Effects of dietary supplementation with vitamin A, D_3, E, and garlic on heat detection, pregnancy rate, and serum profile in anestrus dairy cows

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ABSTRACT

Anestrus poses substantial challenges for dairy industries worldwide. This research aims to evaluate the effect of oral supplementation with garlic alone, vitamins A, D_3, E alone, and their combination on heat detection, pregnancy rate and serum profile in anestrus dairy cows. Using a completely randomized design, this study, conducted over 327 days, evaluated 72 anestrus-affected cows, which were divided into four groups. T_0 (Control) received only normal saline; T_1 with vitamin A,D_3,E; T_2 with Garlic and T_3 integrated with A, D_3, E + Garlic in drinking water. Results demonstrated that the treatment groups exhibited significantly higher estrus signs ($p < 0.05$) compared to the control. The pregnancy rate was also increased ($P = 0.059$), especially in the T_3 group which had the highest heat detection (55.55 %), conception (70 %), and pregnancy (38.88 %). In comparison, the heat detection rate for T_1 was 44.44 %, while the conception rate for T_2 was 66.66 %. Additionally, T_0 exhibited the least proportion across all parameters. Additionally, the elevated BUN (Blood Urea Nitrogen) and blood glucose levels observed in non-responsive (those not showing signs of heat) cows at 32.51 and 43.65 mg/dl respectively, compared to responsive cows (those showing signs of heat) with levels of 22.40 and 51.34 mg/dl. These findings suggest a potential association between these blood parameters and the cyclicity of dairy cows. These findings highlight that oral supplementation with vitamin AD_3E+Garlic significantly improves the heat detection rate and pregnancy rate than only administering vitamin AD_3E or Garlic with anestrus in dairy cattle.

1. Introduction

Anestrus is a condition that refers to animals not showing signs of heat (estrus) because maybe it does not come in estrus, or signs of estrus are not detected. Anestrus is a critical economic issue in the dairy industry resulting in the expansion of non-productive periods. Dairy industries completely rely on regular timely pregnancy, successful breeding, and also milk production. However, anestrus disrupts these crucial processes leading to reduced herd productivity and exacerbating the total farming costs. The disease of genital organs and nutritional imbalance are the two major causes of anestrus. Research showed that the annual rate of culling for dairy herds is mainly due to poor fertility (Guadagnini, Amodeo, Biscarini, Bolli & Moroni, 2023; Kulkarni et al., 2023). Excluding genital infection and managemental faults, nutritional deficiency has a vital role in the reproductive health of cattle.

Vitamins A, E and C act as natural antioxidants, and their presence in nutrition is critical for the prevention of placental retention and other disorders (Didier et al., 2023). Besides, the antioxidant and anti-tumor effects of dietary vitamins A, C, and E have a significant effect on maintaining reproductive health and fertility (Talib et al., 2024; Tous et al., 2014) whereas vitamin E shows several antioxidant functions including trapping peroxyl radicals and protecting polyunsaturated fatty acids from peroxidation (Torsein et al., 2018). Moreover, vitamins such as vitamins B, C, D, E, and some coenzymes play a key role in supporting female reproductive health (Chavarro & Schlaff, 2018). So nutritional dietary supplements draw more attention because of their safety, bioavailability, and efficacy for well-being. Furthermore, vitamins A, D_3, E, and K all have diverse effects on the reproductive health of animals.

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Vitamin A, and β Carotene boost up reproductive efficiency and dietary supplements of vitamin E and selenium reduce incidents of retained placenta and increase conception rate (Ataman et al., 2023). Vitamin A deficiency has a direct effect on the structure and function of the pituitary gland, gonads, and uterus (Zhou, Ma, Shen, Xie & Ren, 2023). A study found that injections of AD_3E and C in combination improved hematological parameters, and immune and antioxidant activities of cattle (Likittrakulwong, Poolprasert, Hanthongkul & Roytrakul, 2022).

A rising number of people are interested in investigating natural dietary supplements as possible therapies for alleviating reproductive difficulties in dairy cows. Garlic or *Allium sativum* is a well-known functional food with several health-promoting qualities. It has shown potential in enhancing animal reproductive outcomes. It has been suggested that adding garlic to dairy cows' diets will improve reproductive metrics like heat detection, pregnancy rate, and serum profile (Assar et al., 2023; Saleh et al., 2023). Garlic is rich in allicin and selenium. Allicin improves blood circulation in the genital organs and protects sperm from being damaged. Allicin, selenium, and other sulfur contained in garlic show immunomodulator, antiparasitic, antimycotic, antiviral, anti-tumoral, and antioxidant effects (Andleeb et al., 2014; Joe, Jayachitra & Vijayapriya, 2009). In addition, the supplementation of dairy cows' diet with garlic combined with organic minerals (Se, Cr, Zn) increased milk production from 12.9 to 20.1 kg and reduced somatic cell count in milk (Prayitno, Suwarno & Jayanegara, 2016). One noteworthy research gap in the dairy industry is the lack of comprehensive research investigating their combined and synergistic effects which is crucial for improving anestrus in the dairy herd. However, further extensive research is mandatory to understand how these substances affect cyclicity, ovarian functions, hormonal balance, and so on.

This study explores the effect of dietary supplementation with vitamins A, D_3, E, and garlic on postpartum estrous in dairy cows. Particularly, we investigate its impact on heat detection, pregnancy rate, and serum profile. Comprehending these associations can disclose targeted interventions to improve fertility and optimize dairy herd health.

2. Materials and methods

This study protocol was reviewed and approved by the Bangladesh Livestock Research Institute (BLRI), Bangladesh (AEEC/BLRI/2023-1027).

2.1. Study area

This study was conducted at Daulatpur Veterinary Hospital, Daulatpur, Khulna, and different farms in the Khulna region in Bangladesh.

2.2. Study period

The study was conducted from 11th January 2023 to 4th December 2023. In this period dairy cows with a history of anestrus were randomly treated with dietary supplements of vitamin A, D_3, E and garlic while another group received normal saline.

2.3. Design of experiment and treatment groups

A total of seventy-two Holstein Friesian cross cows in the age range of 3–6 years with a history of anestrous were treated in this study. Animals with suspected infection of the reproductive system or with a history of abortion were excluded from this study. The study was conducted over a duration of 327 days using a completely randomized design. Three treatments were randomly prescribed in three groups, each consisting of eighteen cows, and another 18 were treated as control groups. The design of the experiments is shown in Table 1.

Each animal was treated for a maximum of 21 days with proper follow-up. After 21 days, animals nonresponsive to treatments were treated with other required treatments (counted as non-responsive animals). If any animal exhibited signs of heat within 21 days, it was inseminated artificially and pregnancy was diagnosed 60–70 days after artificial insemination using rectal palpation. For artificial insemination, 75% Holstein Friesian frozen-thawed semen was used.

Table 1
Experimental design.

Treatment	Number of animals	Notation
Normal saline	18	T_0
A, D_3, E	18	T_1
Garlic	18	T_2
A, D_3, E + Garlic	18	T_3

Here, Treatment T_0 denotes Normal Saline, T_1 denotes AD_3E, T_2 denotes Garlic and T_3 denotes A, D_3, E + Garlic.

Control group (T_0): Each farmer was supplied with around 2000 ml of normal saline (without labeling) and suggested to add 15–20 ml saline in the drinking water of cows for successive 21 days.

A, D_3, E group (T_1): The ingredients of the supplement administered to the T_1 group are shown in Table 2**.** For every 100 kg of body weight, these supplements contained 200 mg of vitamin E, 200,000 IU of vitamin D_3, and 500,000 IU of vitamin A.

Garlic group (T_2): For every 100 kg of body weight, garlic incorporated in water 25 gm.

A, D_3, E group + Garlic group (T_3): This treatment group was the combination of A, D_3, E and Garlic. These supplements contain 500,000 IU of vitamin A, 200,000 IU of vitamin D_3, and 200 mg of vitamin E, and 25 gm- garlic (Table 2**).**

2.4. Diet

In this study, patient owners were suggested to supply a common commercial feed to cattle and not to give any other nutritional supplement. The nutritional value of the commercial feed is given in Table 3**.**

2.5. Data collection

Initial data was collected from owners at the clinics using a questionnaire that recorded the breed, age, number of calvings, duration of infertility, history of abortion, history of endometritis, feeding history, previous treatments, treatments administered, date of onset of treatment, and the treatment outcome (Positive/Negative) after 21 days. Additionally, and date of onset of heat/Conception (If result is positive) were also recorded. Animals are kept under monitoring during the treatment period and up to confirmation of pregnancy by 75 days.

2.6. Biochemical analysis

For the biochemical test, three blood samples were collected from

Table 2
Composition of supplement for Control group (T_0), AD_3E group (T_1), Garlic group (T_2), and AD_3E group + Garlic group (T_3).

Treatment group /Supplement	Dose / 100 kg body weight
Control group (T_0)	15–20 ml 0.9 % Isotonic Saline
A, D_3, E group (T_1)	
Vitamin A	500,000 IU
Vitamin D_3	200,000 IU
Vitamin E	200 mg
Garlic group (T_2)	
Garlic	25 gm
A, D3, E group + Garlic group (T_3)	
Vitamin A	500,000 IU
Vitamin D_3	200,000 IU
Vitamin E	200 mg
Garlic	25 gm

Here, IU = International Unit, mg = Milligram, gm = Gram.

Table 3
Nutritive values of supplied feed.

Chemical composition	Percentage (%)
Humidity (Max)	12
Protein (Min)	16
Fiber (Max)	11
Crud fat (Min)	4
Calcium (Min)	1.2
NFE (Min)	48

Here, NFE= Nitrogen Free Extract.

each group. Using serum samples, blood urea nitrogen (BUN), glucose (GLU), albumin (Alb), triglycerides (Tg), Cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), calcium (Ca), phosphorus (P) and magnesium (Mg) were assayed using an automated biochemical analyzer (Humalyzer-3000®, Guatemala, Germany) according to the prescribed protocol given by the kit manufacturer. Every final group (T_1, T_2, T_3, etc.) had three randomly selected blood samples taken for biochemical examination, except the cows that returned to heat under the control group (which had only two samples) (Fig. 1).

2.7. Statistical analysis

Data from the study was initially entered into Microsoft Excel 2010. Subsequently, the data was transferred to IBM SPSS Statistics 29.0. The study employed a one-way ANOVA test to evaluate the difference between the three treatment groups and the control. Statistical significance was set at a level of $p < 0.05$, indicating that a variable was considered significant if its p-value fell below this threshold.

3. Result

3.1. Heat detection, conception rate and pregnancy rate

In Table 4, the treatment had a significant effect on the heat detection rate across different groups. The highest heat detection rate was observed in T_3, with a rate of 55.55 %, followed by T_1 (44.44 %), T_2 (33.33 %) and the control group T_0 (11.11 %).

The conception rate was highest in group T_3 (70 %), followed by the Garlic group (T_2) at 66.66 %. Besides, 62.50 % conception rate was observed in group AD_3E and the lowest conception rate was in the control group (50 %) (Table 4).

3.2. Biochemical test of serum

After analysis of blood parameter of animals that comes in heat after treatment period, it was found that LDL levels was significantly higher ($P < 0.05$) in T_3 group than control animals. Cholesterol level was also found significantly higher ($P < 0.05$) in T_3 group than control (Table 5).

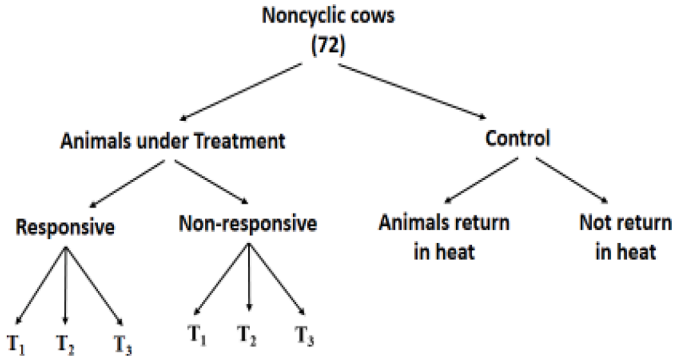

Fig. 1. Three blood samples were collected randomly from each of the final groups (T_1, T_2,T_3 etc.

Table 4
Heat rate, Conception rate and pregnancy rate and p value. Values followed by (*) represent significant differences of $P \leq 0.05$.

Consideration	Treatments				*P*
	T_1 (%)	T_2 (%)	T_3 (%)	T_0 (%)	
Heat detection rate	44.44	33.33	55.55*	11.11	0.036
Conception rate	62.50	66.66	70	50	0.96
Pregnancy rate	27.22	22.22	38.88*	5.55	0.059

Here, Treatment T_0 denotes Normal Saline, T_1 denotes AD_3E, T_2 denotes Garlic and T_3 denotes A, D_3, E + Garlic, Significant ($p \leq 0.05$). The asterisk (*) indicates statistical significance, especially when comparing different treatments or groups.

Table 5
Blood biochemical parameter of responsive (animal showed signs of heat) cow after 21 days of treatment (Mean + SE).

Treatments	T_1	T_2	T_3	T_0	*P*
BUN	19.52 ± 0.98	24.59 ± 0.63	23.08 ± 0.05	18.61 ± 0.38	0.110
Glucose	53.45 ± 1.15	57.23 ± 2.30	48.67 ± 1.62	46.01 ± 1.46	0.073
ALB	2.73 ± 0.47	2.39 ± 0.28	3.11 ± 0.84	2.76 ± 0.51	0.562
TG	15.26 ± 0.88	14.93 ± 0.93	15.31 ± 1.12	12.16 ± 0.63	0.481
HDL	117.81 ± 2.73	112.33 ± 2.34	120.11 ± 1.78	115.76 ± 1.80	0.268
LDL	83.18 ± 2.03	82.26 ± 1.82	98.93* ± 1.52	68.05 ± 1.45	0.019
Cholesterol	122.56 ± 2.23	129.71 ± 3.50	139.01* ± 3.34	128.78 ± 1.61	0.050
Calcium	7.86 ± 0.37	8.25 ± 0.87	7.45 ± 0.42	8.74 ± 0.57	0.065
Magnesium	6.75 ± 0.64	7.21 ± 0.41	7.07 ± 0.52	7.81 ± 0.13	0.499
Phosphorus	8.32 ± 0.71	7.86 ± 0.45	7.91 ± 0.34	7.62 ± 0.26	0.871

Here, Treatment T_0 denotes Normal Saline, T_1 denotes AD_3E, T_2 denotes Garlic and T_3 denotes A, D_3, E + Garlic, BUN = Blood Urea Nitrogen, ALB= Albumin, TG= Triglycerides, HDL= High-Density Lipoprotein, LDL= Low-Density Lipoprotein, The asterisk (*) indicates statistical significance, especially when comparing different treatments or groups. Significant ($p \leq 0.05$).

In the biochemical parameter of non-responsive cows (cows did not show signs of heat) after the treatment period, triglyceride levels were found to be significantly lower ($P < 0.05$) in the T_3 group compared to the control group, while glucose levels were relatively higher. Both BUN and glucose levels were also relatively higher in T_1 group compare to the control (Table 6) alongside no significant differences are found among other parameters.

4. Discussion

The study revealed that the heat detection rate was significantly higher in the treatment groups, particularly in the T_3 group compared to the control group. This indicates that the cows' reproductive profiles were positively influenced by the vitamin A, D_3, and E supplements, aligning with the findings of Likittrakulwong et al. (2022). Additionally, garlic supplementation also seems to have an antibacterial effect on the reproductive system, potentially reducing subclinical endometritis and thereby improving reproductive activity. According to Dvořáková, Weingartová, Nevoral, Němeček and Krejčová (2015), the selenium content of garlic may also have contributed to better reproductive outcomes. These findings highlight the potential benefits of these dietary supplements in improving reproductive efficiency in cows.

The conception rate was around 66% in treatment group whereas in control group rate is 50 %. It was obvious that vitamin A, D, E and garlic

Table 6
Blood biochemical parameter of non-responsive (animal did not show signs of heat) cow after 21 days of treatment (Mean + SE).

Treatments	T_1	T_2	T_3	C	*P*
BUN	36.85 ± 0.81	31.59 ± 0.42	31.74 ± 0.78	29.86 ± 0.56	0.401
Glucose	45.11 ± 1.21	37.32 ± 1.22	53.34 ± 1.03	48.45 ± 2.46	0.063
ALB	3.09 ± 0.08	2.86 ± 0.42	2.44 ± 0.17	1.92 ± 0.39	0.844
TG	18.93 ± 0.48	15.59 ± 0.64	13.30* ± 1.56	17.97 ± 0.92	0.044
HDL	121.14 ± 2.16	126.04 ± 1.16	122.45 ± 2.69	124.12 ± 3.22	0.964
LDL	79.84 ± 1.12	78.92 ± 1.71	88.93 ± 2.83	83.34 ± 1.83	0.548
Cholesterol	161.51 ± 2.01	153.04 ± 3.95	162.34 ± 2.77	149.30 ± 3.74	0.789
Calcium	9.11 ± 0.41	8.71 ± 0.32	9.34 ± 0.22	8.01 ± 0.35	0.634
Magnesium	7.89 ± 0.44	7.97 ± 0.22	7.78 ± 0.35	8.04 ± 0.32	0.954
Phosphorus	7.88 ± 0.18	8.08 ± 0.48	7.75 ± 0.58	8.91 ± 0.39	0.871

Here, Treatment T_0 denotes Normal Saline, T_1 denotes AD_3E, T_2 denotes Garlic and T_3 denotes A, D_3, E + Garlic, BUN= Blood Urea Nitrogen, ALB= Albumin, TG= Triglycerides, HDL= High-Density Lipoprotein, LDL= Low-Density Lipoprotein; The asterisk (*) indicates statistical significance, especially when comparing different treatments or groups; Significant ($p \leq 0.05$).

relatively increase conception rate agreed with the result of Yildiz (2016).

There is a significant difference between the T_3 and control group, with the T_3 group showing a higher pregnancy rate compared to both the T_1 and T_2. Administering vitamin, A, D, E stimulate rapid uterine involution and high pregnancy rate stated by Abdelhameed, Ahmed, El-Ekhnawy and El-Khadrawy (2009). Pregnancy rate or conception rate both increased because of vitamin A, D, E supplement as well as selenium content in garlic, all have effect on functioning of pituitary gland, gonads, and uterus.

Blood parameter of both responsive (showing sign of heat after treatment) and non-responsive (not showing sign of heat after treatment) cows were analyzed, where BUN of responsive animal to treatment was found lower than non-responsive animals. Blood glucose is relatively high in animals those responded to treatment especially in T_2 group. This result has coherence with the findings of Barson et al., 2019; Likittrakulwong et al., 2022; Pathan et al., 2011.

Glycogenolysis and hyperinsulinemia are the two main reasons for low glucose level in non-cyclic animals (Mukherjee, Carroll & Matfin, 2011). Higher glucose level and lower BUN in blood serum of responsive animals may related to garlic containing selenium, vit. A, B, C and E (Asadi et al., 2021) or vitamin A, D_3, E supplements which act as antioxidants and improve blood circulation (Omur et al., 2016).

Albumin level in blood serum was found almost close in both responsive and non-responsive animals to treatments, whereas triglycerides levels in non-responsive animals were found significantly lower in T_3 group than in control group. Although triglycerides have no relation to reproductivity but excess calories in diet causes high concentration of triglycerides in blood serum. Vitamin A, D_3, E also have the effect of low albumin in blood serum stated by Avcı, Küçükkurt, Kontaş, Eryavuz and Fidan (2013).

Increased cholesterol levels were observed in the treatment groups of both responsive and non-responsive animals. LDL concentrations were significantly higher in treatment groups than in control particularly in responsive animals. The serum cholesterol of treatment group of animals was significantly higher than control group and cholesterol level of non-responsive animal was higher than responsive animal which was similar to the result of Tasnim, Ahammed, Mahfuz, Hasan and Shawan (2018) unlike the result of Zhang et al., 2021. According to Li et al. (2021), vitamin D supplement reduce cholesterol and LDL, which is a disagreement with this finding. In this study there was less difference in HDL concentration between treatment and control group.

Level of magnesium and phosphorus were not significantly different between treatment and control group but calcium level in control group which responded to treatment were relatively higher than treatment group. Both T_1 and T_3 groups supplied with vitamin D, found less calcium level than control group disagreed with the findings of Mahen, Williams, Smith and Grove-White (2018). This is likely due to the negative correlation between ionized calcium levels in the blood and time to conception (Beck et al., 2022). Since more animals in the treatment group conceived compared to the control, this resulted in a lowers calcium levels in the treatment group.

5. Conclusions

Heat detection and pregnancy rates are remarkably increased in after Oral application of garlic with vitamin. Significant variances in blood parameters were seen when comparing animals that responded to the treatment with those that did not. However, the effect of different concentration of vitamin supplement with garlic as well as different form of administration need to investigate further to validate these findings and evaluate their impact on blood parameters also.

Ethical statement

This study protocol was reviewed and approved by the Bangladesh Livestock Research Institute (BLRI), Bangladesh (AEEC/BLRI/2023-1027).

CRediT authorship contribution statement

Shaolin Ferdouse: Resources, Methodology, Funding acquisition, Conceptualization. **Md. Shahadat Hossain:** Supervision, Software, Methodology, Investigation. **Rumon Talukder:** Visualization, Validation, Resources. **Md. Asaduzzaman Lovelu:** Project administration, Investigation. **Nahid Rahman:** Supervision, Conceptualization. **Sabuj Kanti Nath:** Writing – review & editing, Writing – original draft, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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