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Correlation of blood biochemical constituents with phases of estrous cycle in Kangayam cows following adoption of estrus induction protocols

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Abstract
Blood biochemical parameters in Kangayam cows would provide a baseline data for future infertility diagnostic reference. Apparently healthy Kangayam cows were subjected to estrus induction protocols and fixed time artificial insemination to correlate with the follicular (day of artificial insemination) and luteal (10 days post insemination) phases of the estrous cycle. Non pregnant, pluriparous Kangayam cow (n=70) were equally allotted to control, and different treatment protocols based on the transdermal (PNC based groups) and transvaginal application of progesterone (CIDR based group) with a shot of $PGF_{2}\alpha$ one day preceding the day of progesterone removal. Ovulation was induced either using estradiol benzoate or buserelin acetate. Haematological and biochemical profiles were analysed on the day of progesterone application (APP), removal (REM), AI and 10 days post insemination (10 DPAI). Results indicated that most of the biochemical parameters were high on 10 DPAI than the day of AI. Hence, it was concluded that the changes between the treatment days could be related to the requirement of energy and protein during the follicular and luteal phases of estrous cycle in Kangayam cows.

Keywords: Kangayam cow, biochemical profile, estrus induction, *Bos indicus*

Introduction
Kangayam cattle is one of the cattle breeds found in Tamil Nadu and it is very prestigious to rear this breed of cattle in this state. Metabolic profiling refers to the analysis of blood biochemical parameters that are useful to assess and prevent metabolic and nutritional disorders. Information on the metabolic indicators could be used to evaluate the energy and protein imbalance with the diet available to the cows (Rossato *et al*., 2001) [15]. But such information (Periyannan, 2021) [12] is scarce for this prestigious indigenous breed of cattle. These cows are reared in subtropics where the availability of succulent green fodder is limited throughout the year. Information on the blood biochemical parameters like energy and protein parameters in the active age group of reproduction would provide us the valuable data like impact of these parameters on reproduction, as well as improving its productivity and health status.

Kangayam cattle is a well-known draught breed, and its milk production is very low (Pattabiraman, 1958) [11]. Nowadays, because of the availability of agricultural machinery, use of this breed of cattle for agricultural activities is becoming less. At the present scenario this breed of cattle is reared for milk, milk products especially ghee; waste products of these cattle are used to make panchakavya, amirthakarisal and other agricultural growth promoters and insecticides. Also, waste products of these cattle are used to make sacred ash, agarbatti, sambarani and even cosmetic products like soaps etc. Hence, rearing this breed of cattle is slowly gaining momentum. Hence, this present work of biochemical profiling using apparently normal healthy cows reared at field conditions and are capable of reproducing was used to obtain the values of certain biochemical parameters. Changes in the levels during different treatment days were studied to correlate with the follicular and luteal phases.

Materials and Methods

Estrus induction protocols were performed in the non pregnant, pluriparous Kangayam cows (n=70) which are reared in and around Tirupur district. All the cows were supplemented with mineral mixture 50g/day for 20 days prior to the estrus induction procedure. Cows in control group were performed with AI, twice at 24 hours interval in the observed estrus after mineral mixture supplementation for 20 days. Estrus induction was carried out using the progesterone hormone either using controlled internal drug releasing device (CIDR based group) through transvaginal application or ProSync-NC (purchased from TRPVB a constituent laboratory under TANUVAS [PNC based group]) through transdermal application (Lakshmikantan *et al*., 2021) [6] for 7 and 5 days respectively. On the preceding date of progesterone removal 500 µg of cloprostenol sodium was injected in the treated cows. For the induction of ovulation, either estradiol benzoate 1 mg (CIDR-EB and PNC-EB groups) 24 hours before AI or buserelin acetate 10 µg (CIDR-G and PNC-G groups) at AI was used. Blood samples were collected at progesterone application (APP), removal (REM), AI and 10 days post AI (10 DPAI) in both clot activator vials (5 ml) and in 5 ml EDTA vials. The samples were analysed for hemoglobin (g/dl) and biochemical parameters like random blood sugar (mg/dl), blood urea (mg/dl), total cholesterol (mg/dl), triglyceride (mg/dl), total proteins (g/dl) albumin (g/dl) globulin (g/dl), albumin globulin ratio. Results were analysed using one way ANOVA and if the ALPHA level estimated was less (≤0.05) then bonferroni correction was carried out and student t test (post hoc analysis) was conducted to identify the significant differences between the treatment days in each group using Microsoft Excel 365.

Results and Discussion

Estrus induction was earlier attempted in Kangayam cows (Manokaran *et al*., 2023) [8]. In the present study along with the estrus induction protocols it was planned to consider the biochemical changes that occur during the different days of treatment. Haemoglobin estimation and biochemical parameter analysis was planned to mimic either the follicular or luteal phase in different treatment days. Hence, the following days of treatment were selected *viz*., at the initiation progesterone treatment (in which the cows selected for the study could be at different reproductive status because treatment protocols were initiated irrespective of the stage of estrous cycle), at the time of progesterone removal i.e. one day after the administration of luteolytic agent, cloprostenol sodium, on the day of AI and then the 10th day post AI (10 DPAI). In which at the time of progesterone application some cows were in anestrus so the values obtained may or may not reflect either follicular or luteal phase. Even though progesterone was supplemented through external source, the day of removal and the day of AI, both could be considered for the follicular phase of the estrous cycle because impact of the corpus luteum is minimal or nil in these days respectively. 10 DPAI could be considered as the luteal phase. Levels of haemoglobin and other biochemical parameters in different groups – control and treatment groups were provided in Table 1 to 7. Table 8 shows the values of haemoglobin and other biochemical parameters in all the cows selected for the study irrespective of the treatment protocol adopted.

In the hematological parameters hemoglobin was considered as this study was carried out in field and these animals are routinely sent for grazing which might increase the external parasite encounter and would have affected the health status of the cows, as it could reduce the level of hemoglobin. However, in all the cows studied in various treatment and control groups, the level of hemoglobin was between 10.97 + 0.41 g/dl and 12.50 + 0.49 g /dl; and in every treatment group levels between the days of treatment did not differ statistically. So, there were no significant changes in the hemoglobin levels in different treatment days in each treatment protocol adopted.

Blood sugar levels could be used to get an idea of dietary adequacy and ruminants could synthesize glucose in liver from volatile fatty acids (Lager and Jordan, 2012) [5]. Velladurai *et al*. (2014) [18] stated that blood sugar is a metabolic signal providing information for the external control of GnRH release and also reported that expression of estrus at first postpartum ovulation was more likely in cows which maintained at higher glucose levels. Sugar levels in this study were high on the day of 10 DPAI than the AI in control, PNC based treatment groups and in all the cows under study irrespective of the treatment groups but in CIDR based treatment groups the levels were low, and it was significantly low in the CIDR group.

Blood urea concentration was also having the same pattern between PNC and CIDR based treatment groups even though there were no significant differences observed. Van Saun (1997) [17] stated that blood urea levels could be under the influence of dietary protein intake, amino acid composition of the diet, liver and kidney function, muscle tissue breakdown, dietary carbohydrate level and rumen degradability. Law *et al*. (2009) [7] indicated that increased blood urea may be due to increased ammonia detoxification in the liver. Park *et al*. (2010) [10] reported that high blood urea level could indicate lipo mobilization. Since, cows under field conditions were not fed adequate concentrate diet, to meet the energy requirement of the cow in luteal phase there might be chances of lipo mobilization that increases the blood urea level. Roche *et al*. (2000) [14] stated that increased blood urea in postpartum cows when fed high protein diet had lower fertility indicating high levels of blood urea is deleterious with respect to fertility. In the present study cows are maintained by the farmers majorly by grazing in which the supply of excess protein may not be possible and hence lipo mobilization could be the reason for variations in the blood urea levels.

Cholesterol is the precursor for steroid hormones. Radkowska and Herbut, (2014) [13] reported that cholesterol levels were low in the cows in which pasture availablity is more. The levels of cholesterol were high on 10 DPAI than on the day of AI in majority of the groups which was in concurrence with the findings of Choudhary *et al*. (2018) [4], Periyannan (2021) [12] and Selvaraju and Ganesh (2022) [16] except CIDR and CIDR-EB groups. It is noteworthy that CIDR-G group was different from other CIDR groups. This gives the suspicion that GnRH treatment at the time of AI might have some influence on the level of cholesterol which was not in agreement with the findings of Bhoraniya *et al*. (2012) [1] and Naikoo *et al*. (2021) [9]. These variations in cholesterol levels were not significant statistically except in control and in all the cows under study irrespective of the treatment protocol adopted.

In the energy metabolism the next important compound is triglyceride which is converted into very low density lipoprotein (VLDL) in the liver of the cow and are involved in energy production. These triglyceride levels were high on the day of 10 DPAI than the day of AI in all the groups. This result was in accordance with the findings of Velladurai *et al*. (2014) [18] in crossbred cows; Bhoraniya *et al*. (2012) [1] in

Kankrej cows; Selvaraju and Ganesh (2022) [16] in buffaloes. However, Naikoo *et al*. (2021) [9] showed such increase on 10 DPAI was only observed in the treatment with CIDR combined with Ovsynch protocol. In the present study variations observed between the treatment days regarding energy related compounds like blood sugar, cholesterol, triglyceride levels reveal that there was certain pattern; like changes between PNC and CIDR based groups raises the question that method of progesterone administration either through transdermal or transvaginal might influence these parameters, specifically random blood sugar and blood urea, however this statement requires further confirmation.

Total protein levels were varying between days of treatment especially AI and 10 DPAI in which levels were high on 10 DPAI in the following groups *viz*., control, PNC, CIDR, PNC-EB and CIDR-EB and It was low or there was not much variation in PNC-G and CIDR-G group respectively. Cevik *et al*. (2010) [3] stated that proteins are reported to be utilised for the preparation of histotropic nutrition for the developing embryo from the uterus. In GnRH treated groups (PNC-G and CIDR-G groups) low protein level in the plasma indicate that such utilisation is more in these treatment groups which could be beneficial for the early embryo development. In accordance with the present results Choudhary *et al*. (2018) [4]; Selvaraju and Ganesh, (2022) [16] stated that protein levels were higher during luteal phase in Gir cows and buffaloes respectively.

As for as the albumin levels are concerned, the changes were not significant except CIDR group. In this group there were significant statistical difference noticed between the days of AI and 10 DPAI. However, the changes in other treatment groups are very less even though there were higher levels in 10 DPAI than the day of AI. Burke *et al*. (2010) [2] reported that endometritis at 42 days postpartum was associated with lower concentration of albumin and lower albumin globulin ratio in cows indicating its role in the occurrence of uterine infections.

Globulin values were also high on the day of 10 DPAI except PNC-G group. However, there was no statistical significance observed in any of the groups. In all the groups considering albumin globulin ratio, the values were not varying between treatment days. But these parameters have their significance in the occurrence of uterine infections (Burke *et al*. 2010) [2].

The levels of energy and protein parameters were generally high on the day of 10 DPAI than the day of AI. Even though there were certain differences between PNC based and CIDR based treatment groups, however usefulness of such differences was not clear. GnRH administration at the time of AI had some influence especially on the energy parameters at the time of luteal phase especially cholesterol, and triglyceride and also on the total protein levels. Variations in blood glucose, cholesterol, triglyceride and also in total protein during the treatment days in different treatment groups indicated that these components were utilised for the energy and protein requirements of the cow and blood urea could be used as the indirect measurement of lipo mobilization to match the energy requirement of the cow at the situation of low energy supplemented as in the field conditions.

Hence, it could be concluded that biochemical parameters are varying between treatment days especially on the day of AI and 10 DPAI that could be related to the requirement of energy and protein and the levels also vary depending on the stage or phase of the estrous cycle in Kangayam cows.

Table 1: Biochemical values in control group on the day of AI and 10DPAI

CON	Hb g/dl	Random blood sugar mg/dl	Blood urea mg/dl	Total Cholesterol mg/dl	Serum Triglyceride mg/dl	Proteins (Total) g/dl	Albumin g/dl	Globulin g/dl	A/G ratio
AI	11.22±0.42	71.17±2.18	16.82±1.39	115.97±2.85[a]	26.92±1.10[a]	7.07±0.13	3.18±0.05	3.88±0.13	0.83±0.03
10DPAI	11.80±0.45	76.33±3.54	19.78±1.9	131.80±3.36[a]	35.48±2.73[a]	7.40±0.20	3.28±0.07	4.12±0.19	0.81±0.04

Same superscripts differ significantly within the column (P≤0.01)

Table 2: Biochemical values in PNC group during progesterone based estrus induction protocols

PNC	Hb g/dl	Random blood sugar mg/dl	Blood urea mg/dl	Total Cholesterol mg/dl	Serum Triglyceride mg/dl	Proteins (Total) g/dl	Albumin g/dl	Globulin g/dl	A/G ratio
APP	11.94±0.20	75.14±1.84	20.75±1.73	133.83±08.23	44.55±9.03	7.20±0.23	3.20±0.07	4.00±0.25	0.81±0.06
REM	11.81±0.43	65.14±4.07	20.25±3.13	120.71±04.32	31.02±2.26	7.07±0.22	3.10±0.08	3.97±0.24	0.81±0.08
AI	12.39±0.34	64.71±5.13	19.74±1.84	149.66±16.55	36.55±6.32	7.31±0.08	3.16±0.06	4.16±0.10	0.77±0.02
10DPAI	12.46±0.16	73.57±3.25	26.22±2.03	164.57±18.69	47.91±8.18	7.54±0.22	3.31±0.09	4.23±0.29	0.81±0.07

Table 3: Biochemical values in CIDR group during progesterone based estrus induction protocols

CIDR	Hb g/dl	Random blood sugar mg/dl	Blood urea mg/dl	Total Cholesterol mg/dl	Serum Triglyceride mg/dl	Proteins (Total) g/dl	Albumin g/dl	Globulin g/dl	A/G ratio
APP	11.45±0.24	42.38±2.10[a]	17.91±1.08	141.98±08.34	40.63±3.87	6.99±0.31	3.29±0.04[a]	3.70±0.30	0.92±0.06
REM	11.36±0.27	67.38±1.07[abc]	21.51±1.91	126.56±02.79	30.23±1.14	7.34±0.13	3.14±0.07[b]	4.20±0.13	0.75±0.03
AI	12.03±0.30	43.75±3.00[b]	22.11±0.94	145.11±10.51	36.13±5.36	7.28±0.22	3.30±0.09[c]	3.98±0.27	0.87±0.08
10DPAI	11.85±0.35	36.88±2.39[c]	21.46±0.78	138.56±02.55	38.88±4.28	7.60±0.17	3.66±0.12[abc]	3.94±0.13	0.94±0.04

Same superscripts differ significantly within the column (P≤0.01)

Table 4: Biochemical values in PNC-EB group during progesterone based estrus induction protocols

PNC-EB	Hb g/dl	Random blood sugar mg/dl	Blood urea mg/dl	Total Cholesterol mg/dl	Serum Triglyceride mg/dl	Proteins (Total) g/dl	Albumin g/dl	Globulin g/dl	A/G ratio
APP	12.10±0.33	78.86±3.07	21.23±2.86	143.09±04.33	54.23±8.95	7.24±0.21	3.26±0.08	3.99±0.17	0.83±0.03
REM	11.93±0.43	71.29±2.93	18.45±1.68	132.88±05.40	31.82±2.18	7.16±0.18	3.29±0.10	3.87±0.12	0.85±0.04
AI	12.13±0.27	72.14±3.17	16.56±1.63	160.14±18.26	37.37±4.10	7.37±0.22	3.17±0.09	4.20±0.23	0.77±0.05
10DPAI	12.11±0.25	73.57±1.27	21.77±2.12	158.21±11.99	50.62±8.28	7.80±0.41	3.37±0.12	4.43±0.48	0.80±0.08

Table 5: Biochemical values in CIDR-EB group during progesterone based estrus induction protocols

CIDR-EB	Hb g/dl	Random blood sugar mg/dl	Blood urea mg/dl	Total Cholesterol mg/dl	Serum Triglyceride mg/dl	Proteins (Total) g/dl	Albumin g/dl	Globulin g/dl	A/G ratio
APP	11.92±0.89	80.00±5.49	17.90±2.78	165.62±08.43	52.34±09.25	6.96±0.22	3.38±0.15	3.58±0.21	0.95±0.06
REM	13.38±0.66	76.40±4.87	17.16±2.57	131.48±07.23	25.88±01.03	6.84±0.14	3.28±0.08	3.60±0.21	0.93±0.08
AI	11.88±0.91	70.20±4.09	16.56±1.08	140.54±10.15	38.32±06.94	7.22±0.22	3.36±0.04	3.86±0.19	0.87±0.04
10DPAI	12.30±0.73	68.00±3.39	13.22±1.33	136.08±10.90	44.94±12.66	7.56±0.39	3.32±0.14	4.24±0.36	0.81±0.08

Table 6: Biochemical values in PNC-G group during progesterone based estrus induction protocols

PNC-G	Hb g/dl	Random blood sugar mg/dl	Blood urea mg/dl	Total Cholesterol mg/dl	Serum Triglyceride mg/dl	Proteins (Total) g/dl	Albumin g/dl	Globulin g/dl	A/G ratio
APP	11.40±0.52	73.67±1.41	22.40±3.27	155.68±09.27	50.73±5.39	7.02±0.41	3.23±0.03	3.78±0.41	0.91±0.09
REM	12.40±0.48	68.33±2.96	20.05±2.86	123.62±03.95	31.33±2.55	7.08±0.24	3.22±0.05	3.87±0.26	0.84±0.06
AI	11.82±0.48	68.00±5.70	18.52±1.36	143.17±14.38	45.93±8.60	7.17±0.14	3.15±0.10	4.02±0.10	0.78±0.04
10DPAI	12.03±0.64	75.17±2.18	19.00±0.83	166.68±21.72	48.15±6.41	6.65±0.20	3.23±0.05	3.42±0.18	0.95±0.04

Table 7: Biochemical values in CIDR-G group during progesterone based estrus induction protocols

CIDR-G	Hb g/dl	Random blood sugar mg/dl	Blood urea mg/dl	Total Cholesterol mg/dl	Serum Triglyceride mg/dl	Proteins (Total) g/dl	Albumin g/dl	Globulin g/dl	A/G ratio
APP	10.97±0.41	72.00±4.73	14.68±1.61	126.00±07.22	57.27±7.46	7.20±0.29	3.42±0.09	3.78±0.32	0.94±0.09
REM	12.50±0.49	71.33±3.62	16.70±2.57	120.67±07.96	26.83±3.94	6.85±0.23	3.25±0.04	3.60±0.25	0.93±0.07
AI	11.78±0.57	70.33±3.84	16.75±1.21	115.52±06.05	33.05±8.90	7.20±0.29	3.33±0.10	3.87±0.24	0.88±0.06
10DPAI	11.47±0.46	70.67±2.63	15.65±2.03	136.43±12.01	39.6±9.730	7.27±0.17	3.28±0.10	3.98±0.11	0.81±0.03

Table 8: Biochemical values in all the cows selected for the study irrespective of the treatment protocol adopted

ALL GROUPS	Hb g/dl	Random blood sugar mg/dl	Blood urea mg/dl	Total Cholesterol mg/dl	Serum Triglyceride mg/dl	Proteins (Total) g/dl	Albumin g/dl	Globulin g/dl	A/G ratio
APP	11.63±0.17	69.25±2.49	19.24±0.93	143.63±3.49^{a}	50.19±3.03^{ab}	7.10±0.11	3.28±0.03	3.82±0.11	0.89±0.03
REM	12.09±0.20	69.23±1.37	19.21±0.96	126.72±2.23^{ab}	29.87±0.94^{acd}	7.08±0.08	3.21±0.03^{a}	3.88±0.08	0.84±0.02
AI	11.88±0.17	64.91±2.10	18.33±0.59	140.22±5.23	37.02±2.47^{bc}	7.23±0.07	3.23±0.03	3.99±0.08	0.82±0.02
10DPAI	12.01±0.16	66.80±2.35	19.97±0.83	147.89±5.12^{b}	44.41±2.93^{d}	7.41±0.11	3.35±0.05^{a}	4.05±0.11	0.84±0.02

Same superscripts differ significantly within the column ($P \leq 0.01$)

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