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RESEARCH ARTICLE

INTERVENTIONAL METAPHYLAXIS AND UNDESIRABLE EFFECTS OF EXCEDE (CEFTIOFUR) AGAINST PNEUMONIA IN DAIRY CALVES UNDER EXTREME ENVIRONMENTAL CONDITIONS.

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Abstract

Biosecurityin dairy farms depends on a broad range of preventive measures that are fundamental to optimal calf health from birth to weaning. Calf hood diseases have a major impact on the economic viability of cattle operations. Metaphylactic effect of Excede (Ceftiofur crystalline free acid) was evaluated in 30 dairy calves under cold stress for a period of 5 wks; during which a total of 450 samples (150 whole blood, 150 serum and 150 nasal swaps) were collected and examined (hematological - Biochemical and Bacteriological). Some of the injected animal exhibited few signs of pneumonia with morbidity rate up to 33.3% in injected groupand 13.3 % in control group. A highly significant decrease ($P < 0.01$) in WBCs and Globulin, a highly significant increase ($P < 0.01$) in Albumin, A/G ratio; ALT; Glucose and log TPC confirmed the development of stress. Temperature revealed a significant strong negative correlation with WBCs count, a significant strong positive correlation with Albumin; a highly significant ($P < 0.01$) strong positive correlation with ALT and a significant intermediate negative correlation with Creatinine. Relative humidity revealed a significant intermediate negative correlation with hemoglobin concentration; a significant strong negative correlation with log TEC; a highly significant strong negative correlation with MCHC and WBCs count; a significant intermediate positive correlation with Albumin; a significant ($P < 0.01$) strong positive correlation with ALT and a significant intermediate negative correlation with Creatinine. In conclusion; a failure of Excede (Ceftiofur) was certified to perform a promising metaphylaxis in dairy calves; although the undesirable effects were developed under the mercy of adverse environmental conditions and contributed an increase in the overwhelming challenges on calves.

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Introduction:-

Temperature and relative humidity incriminated in host behavioral changes as more time spent outdoors or in closed environments during cold or rainy weather, also causes deviations in host defense mechanisms as mucociliary clearance impairment on inhalation of cold, dry air, and changes in the microbial infectivity and stability **(Pica andBouvier, 2012)**. Calves are especially susceptible to the negative effects of cold due to having a larger surface area to body mass ratio than more mature animals, resulting in more body heat loss with the larger surface area (**Ames and Insley, 1975).** Cold stress causes calves' energy to be used for maintenance rather than being utilized elsewhere in the body.

In cold months, pneumonia contributed high morbidity and mortality resulting in considerable economic losses through reduction of weight gain, treatment costs and production losses **(Sayed and Zaitoun, 2009).** Thus pneumonia is considered as one of the most important clinical and economic problem affecting newborn calves. Enzootic bronchopneumonia is primarily a problem of calves less than six months old with peak occurrence from two to ten weeks of age but also may occur in calves up to one year of age. It is more common in dairy calves than in beef one and considered as a common problem in veal calves and the peak incidence of the disease may coincide with decline of passively acquired immunity**(Merck and Merial, 2002).**

Excede Sterile Suspension is a ready-to-use formulation that contains the crystalline free acid of ceftiofur. Ceftiofur is a broad spectrum cephalosporin, highly effective against wide range of veterinary important gram-positive and gram-negative pathogens, including those β-lactamase–producing strains **(Samitz et al., 1996 and Arrioja-Dechert, 2001)**. Ceftiofur is a bactericidal inhibiting bacterial cell wall synthesis by covalently binding to the penicillin-binding proteins (PBPs), which are essential for the bacterial wall synthesis. Ceftiofur is clinically used in the United States and Europe for control of bovine respiratory disease (BRD) caused by *Mannheimiahaemolytica* (previously known as *Pasteurellahaemolytica*), *Pasteurellamultocida*, and *Haemophilussomnus***(FDA, 1988 and 1991)**.

The present study aimed to conduct an intervention study to evaluate the metaphylactic effect of Excede (Ceftiofur crystalline free acid) on the incidence of Pneumonia in dairy calves referring to its possible undesirable effects under adverse environmental conditions (cold stress).

Material and methods:-

The presentexperiment was conducted at El-Salhya Dairy Farm, El-Sharkia, Egypt.

Animals and managements:-

Thirty (30) dairy calves were selected from time of birth on a weight basis (average body weight 30 kg). Animals were divided randomly allocated into two equal groups (15 calves of each); the 1st group (G1) was used as control healthy animals, and the 2nd group (G2) was assigned to be injected with Excede® **(ceftiofur 200 mg/ml)** onceby6.6 mg ceftiofur/kg^{-1} subcutaneously . All calves were housed individually in a separate calf pens on dirty floor system from birth up to 2 weeks of age then, the calves were transferred to a group hutch until the weaning time; the calves were transferred between 2 - 3 group hutches to control the wetting of the dirty floor system.

Calves were fed 3 meals of colostrum (1.5 liter per meal per calf) during the first 3 days of life. At the 4th day up to 2 weeks of age; calves were fed on 3 meals of milk at a rate of 2-3 liter per meal per calf; then fed on milk replacer at rate of 2 liter per meal per calf up to 2 month of age and during these period the calf starter and tap water were available *ad libtum*. The newly borne calves were vaccinated with Nasalgin vaccine during the first few days or life.

Sampling and measurements:-

Samples:-

A total number of 450 samples (150 Whole blood sample; 150 serum samples and 150 nasal swab samples) were collected from the two groups of animals during the entire length of the study. The samples were collected just prior to injection (zero time); one week post injection (P1); two weeks post injection (P2); three weeks post injection (P3), and four weeks post injection (P4). All samples were transported in ice box within 1 hour from collection to the research laboratory.

Environmental Macroclimate:-
The environmental temperature and relative humidity were recorded at each sampling time (Zero time, P1, P2, P3, and P4) using Clock &Hygro-Thermometer, (Boeco Germany, model: BOE 325); Indoor/Outdoor-MIN/MAX Thermometer, (Boeco Germany, model: BOE 325).

Hematological analysis:-
Whole blood samples were collected from jugular vein on sterile ready to use DRY MED EDTA K3 Tubes and were used for Red Blood Cells (RBCs), White Blood Cells (WBCs) and Platelets counts; determination of Hemoglobin concentrations (Hb) and Mean Corpuscular Hemoglobin Concentrations (MCHC) according to the routine hematological procedures adopted by **Feldman et al., (2000).**

Biochemical analysis of serum samples:-
Blood samples were collected from jugular vein into 10 mL dry, clean and sterilized disposable vacutainer plain tubes and were used for obtaining serum. Samples were allowed to clot for 30 to 60 minutes, then centrifuged at 3000 rpm for 20 minutes; a clear non-hemolyzed sera were divided into 4 equal parts in eppendorf tubes then stored at -20 °C until be used for the biochemical analysis **(Coles, 1986)**. Total protein (TP), albumin (ALB), triglycerides (TG), total cholesterol (TC), Glucose, Urea and Creatininewere estimated calorimetrically **(Young, 2001)**.Globulin was calculated as the difference between the total protein and albumin and Albumin / Globulins ratio (A/G ratio) was calculated. Serum enzymatic activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined calometerimetrically**(Young, 1990)**.

Bacteriological examination of swabs:-

Preparation of samples:-
All samples were prepared according to the technique recommended by **APHA (2001).** All swabs were collected on 9 mL physiological saline; and on arrival to the laboratory tenfold serial dilution was carried out. From the original dilution, 1 mL was transferred aseptically to a test tube containing 9ml sterile 0.1% buffered peptone water (w / v) to prepare a dilution of 10^{-1}, then from which tenfold decimal serial dilution up to 10^{-6} were prepared to cover the expected range of samples contamination which could be easily counted.

Total Bacterial Count (TBC):-
It was carried-out by drop Plate Method **(Zelver et al., 1999** and **Herigstad et al., 2001)** using Nutrient agar **(Cruickshank et al., 1975; 1980)**. The inoculated as well as un-inoculated control plates were inverted and incubated at 37 $^{\circ}$C for 24 - 48 h. Counting and calculation from plates showed 30-300 CFU.

Total Pasteurella count (TPC):-
Total *Pasteurellamultocida* count in samples was carried-out by drop Plate Method **(Zelver et al., 1999** and **Herigstad et al., 2001)** using Columbia blood agar supplied with 10% sheep RBCs **(Cruickshank et al., 1975; 1980)**. Inoculated and control plates were incubated at 37 $^{\circ}$C for 24 - 48 h. Counting and calculation from plates showed 30-300 CFU.

Total Enterobacterieacae count (TEC):-
It was done by drop Plate Method **(Zelver et al., 1999** and **Herigstad et al., 2001)** using Eosin Methylene Blue Agar (EMB) **(Cruickshank et al., 1975; 1980)**.Bothinoculated and control plates were incubated at 37 $^{\circ}$C for 24 - 48 h. Counting and calculation from plates showed 30-300 CFU. Five typical colonies were selected and cultured onto MacConkey agar plates (Lab-M), and incubated at 37 $^{\circ}$C for 24 h. Pure colonies were inoculated onto nutrient slant and incubated at 37 $^{\circ}$C for 24 h and kept for further identification.

Statistical analysis:-
Statistical analysis was run through SPSS (version, 20) for windows **Levesque, (2007)**. The obtained data were analyzed statistically using factorial experiments of Analysis of Variance (ANOVA) with general linear model procedures (GLM) for all tested groups, times and their interactions. The mean values, standard error (SE) were calculated by using Microsoft Excel program. The correlation co-efficient was calculated to compare the influence of each measured parameter mean values on each other **Fulekar, (2009)**.

Results:-

During the study period; the injected as well as the control animals were observed for development of clinical manifestations of pneumonia. Some injected animals exhibited few signs of pneumonia such as bilateral mucopurulent nasal discharge, paroxysmal moist cough, rattling sound during auscultation of the trachea, meanwhile, auscultation of lungs revealed different abnormal sound such as exaggerated vesicular sound, crackles and wheezes. Morbidity rate was calculated depending on clinical manifestations developed and it was 33.3% in injected group (5 cases out of 15 injected animals)and 13.3 % in control group (2 cases out of 15 control animals).

Hematological findings of the present study showed non-significant differences in hemoglobin concentration; MCHC and Platelets count at the different sampling times and on a broad comparison between the treated group and control one. Although RBCs showed a non-significant difference at most of the sampling time; it showed overall a highly significant increase ($P < 0.01$) in the treated group when compared to the control group. On the contrary; WBCs although showed a non-significant differences at most of the sampling time; it showed overall a highly significant decrease ($P < 0.01$) in the treated group when compared to the control group **(Table 1)**.

Table 1:-Hematological parameters in Excede injected and control animals.

parameter / *Treatment*		*Hemoglobin (g / dL)*	*R.B.Cs ($x10^6$ / µL)*	*M.C.H.C (g / dL)*	*W.B.Cs ($x10^3$ / µL)*	*Blood platelets ($x10^3$ / µL)*
Grs	**G1**	$6.1^{a} \pm 0.1$	$7.5^{b} \pm 0.18$	$32.3^{b} \pm 0.7$	$6.7^{a} \pm 0.2$	$265.8^{a} \pm 3.6$
	G2	$5.9^{a} \pm 0.1$	$8.2^{a} \pm 0.15$	$32.1^{b} \pm 0.6$	$6.1^{b} \pm 0.2$	$267.2^{a} \pm 3.1$
P value		0.450	0.006	0.001	0.074	0.001
Zero	**G1**	$6.14^{b} \pm 0.2$	$7.9^{ab} \pm 0.3$	$33.1^{a} \pm 0.1$	$7.59^{a} \pm 0.6$	$277.6^{ab} \pm 9.1$
	G2	$6.1^{b} \pm 0.2$	$7.6^{ab} \pm 0.3$	$31.8^{ab} \pm 0.1$	$6.67^{ab} \pm 0.2$	$277.4^{ab} \pm 6.2$
1st wk	**G1**	$6.21^{b} \pm 0.2$	$8.17^{b} \pm 0.5$	$32.7^{b} \pm 0.2$	$6.58^{ab} \pm 0.5$	$251.8^{bcd} \pm 9.8$
	G2	$6.1^{b} \pm 0.2$	$8.5^{b} \pm 0.2$	$32.1^{b} \pm 0.1$	$6.41^{ab} \pm 0.3$	$267.1^{abc} \pm 4.9$
2nd wk	**G1**	$6.38^{a} \pm 0.1$	$7.53^{bc} \pm 0.4$	$32.2^{b} \pm 0.2$	$7.31^{b} \pm 0.4$	$274.9^{ab} \pm 8.7$
	G2	$5.7^{ab} \pm 0.1$	$8.3^{b} \pm 0.4$	$32.1^{b} \pm 0.1$	$5.63^{c} \pm 0.2$	$278.6^{a} \pm 6.4$
3rd wk	**G1**	$5.83^{ab} \pm 0.2$	$7.36^{bc} \pm 0.4$	$31.8^{b} \pm 0.1$	$6.26^{ab} \pm 0.6$	$279.6^{a} \pm 8.8$
	G2	$5.7^{ab} \pm 0.1$	$8.8^{a} \pm 0.4$	$32.3^{b} \pm 0.3$	$5.75^{bc} \pm 0.2$	$253.3^{bcd} \pm 5.8$
4th wk	**G1**	$5.66^{ab} \pm 0.6$	$6.59^{c} \pm 0.3$	$31.7^{ab} \pm 0.1$	$5.93^{bc} \pm 0.3$	$244.9^{bcd} \pm 4.2$
	G2	$6.1^{b} \pm 0.1$	$7.8^{ab} \pm 0.2$	$32.1^{b} \pm 0.1$	$6.26^{ab} \pm 0.2$	$259.4^{abc} \pm 6.8$
P value		0.056	0.154	0.001	0.230	0.001

G1: Control group G2: Excede

Means carrying different superscripts in the same column are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$).

Means carrying the same superscripts in the same column are non-significantly different at ($P > 0.05$).

Biochemical analysis of serum samples (Tables 2 - 3) revealed a highly significant increase ($P < 0.01$) in Albumin (Alb); A/G ratio; Triglycerides (TG); Cholesterol (TC); Glucose and ALT in the treated group (G2) when compared to control group (G1). On contrast, a highly significant decrease ($P < 0.01$) was detected in Globulin; AST and Urea serum levels in the treated group (G2) when compared to control (G1). A non-significant difference was clear in both Total protein and Creatinine in the treated group (G2) when compared to control (G1).

Albumin level revealed a highly significant increase ($P < 0.01$) at zero; P1; P2; P3 and P4 in the treated group **(Table 2)**. Furthermore, Glucose and ALT concentration showed a highly significant increase ($P < 0.01$) at P1; P2 and P3 sampling time in the treated group **(Table 3)**. Meanwhile AST showed a highly significant decrease ($P < 0.01$) at zero; P1 and P4 sampling time among the treated group **(Table 3)**.

Table 2:- Proteinogram, TG and TC levels in serum samples collected from Excede injected and control animals.

parameter / Treatment		Total protein (g / dL)	Albumin (g / dL)	Globulin (g / dL)	A / G ratio	TG (mg / dL)	TC (mg / dL)
Grs	G1	5.8 [a] ± 0.2	1.58 [b] ± 0.1	4.16 [a] ±0.2	0.439 [b] ± 0.137	100.2 [b] ± 2.3	55.2 [b] ± 1.9
	G2	5.6 [a] ± 0.1	2.68 [a] ± 0.1	3.19 [b] ± 0.2	1.298 [a] ± 0.116	112.2 [a] ± 1.9	65.8 [a] ± 1.6
P value		0.773	0.001	0.001	0.001	0.001	0.001
Zero	G1	5.0 [b] ± 0.6	1.3 [d] ± 0.2	3.7[ab] ± 0.5	0.380 [d] ± 0.306	103.7[abc] ± 4.6	44.7 [e] ± 4.2
	G2	5.7 [ab] ± 0.1	2.4[ab] ± 0.2	3.3[ab] ± 0.4	0.833 [c] ± 0.259	112.9[ab] ± 4.8	96.3 [a] ± 3.8
1st wk	G1	6.3 [a] ± 0.5	1.6 [c] ± 0.3	4.7 [a] ± 0.5	0.428 [d] ± 0.306	94.3 [d] ± 4.5	48.9 [de] ± 5.1
	G2	5.9 [ab] ±0.1	2.9 [b] ± 0.3	3 [c] ± 0.3	1.38 [b] ± 0.259	103.4[bcd] ± 4.9	58.1 [cd] ± 2.8
2nd wk	G1	5.8 [ab] ± 0.4	1.3 [d] ± 0.2	4.5 [b] ± 0.4	0.326 [d] ± 0.306	101.8[cde] ± 4.7	48.9 [de] ± 2.1
	G2	5.5 [ab] ± 0.2	2.2[ab] ± 0.2	3.3[ab] ± 0.4	1.22 [b] ± 0.259	103.1[cde] ± 3.2	55.6 [cd] ± 4.2
3rd wk	G1	5.3 [ab] ± 0.3	1.6 [c] ± 0.2	3.7[ab] ± 0.4	0.672 [c] ± 0.306	104.2[abc] ±4.9	77.4 [b] ± 5.5
	G2	5.7 [ab] ± 0.2	2.2[ab] ± 0.2	3.5[ab] ± 0.4	0.812 [c] ± 0.259	118.9 [b] ± 5.2	64.4 [c] ± 3.8
4th wk	G1	6.1 [a] ± 0.2	1.6 [c] ± 0.2	4.5 [b] ± 0.3	0.391 [d] ± 0.306	97.2 [c] ± 2.2	56.8 [cd] ± 4.3
	G2	5.6 [ab] ± 0.1	3.2 [a] ± 0.3	2.4 [d] ± 0.3	2.29 [a] ± 0.259	122.6 [a] ± 5.8	54.7 [cd] ± 3.1
P value		0.554	0.050	0.185	0.053	0.001	0.001

G1: Control group G2: Excede

Means carrying different superscripts in the same column are significantly different at (P ≤ 0.05) or highly significantly different at (P < 0.01).

Means carrying the same superscripts in the same column are non-significantly different at (P >0.05).

Table 3:-Glucose, Liver enzymes, Urea and Creatinine levels in serum samples collected from Excede injected and control animals.

parameter / Treatment		Glucose (mg / dL)	ALT (U / L)	AST (U / L)	Urea (mg / dL)	Creatinine (mg / dL)
Grs	G1	75.9 [b] ± 2.4	22.9 [b] ± 0.5	29.2 [a] ± 0.5	54.3 [a] ± 1.8	0.9 [a] ± 0.03
	G2	94.6 [a] ± 2.1	26.9 [a] ± 0.4	23.7 [b] ± 0.4	46.9 [b] ± 1.5	0.9 [a] ± 0.03
P value		0.001	0.001	0.001	0.010	0.120
Zero	G1	80.9[bc] ± 6.7	21.5 [de] ± 0.7	31.5 [a] ± 1.3	49 [d] ± 4.9	1.1 [a] ± 0.1
	G2	90[ab] ± 4.1	23.3 [d] ± 1.1	18.8 [e] ± 1.1	35.8 [t] ± 1.9	1[ab] ± 0.1
1st wk	G1	76.7 [d] ± 6.8	21.4 [e] ± 0.6	27.3 [c] ± 1.2	55.2 [c] ± 5.2	1.1 [ab] ± 0.1
	G2	113.8 [a] ± 4.9	29.8 [a] ± 1.5	19.2 [e] ± 0.7	52.1[bc] ± 3.2	0.9 [b] ± 0.1
2nd wk	G1	68.5 [de] ± 5.7	23.2 [d] ± 0.9	27.7 [c] ± 0.7	71.9 [a] ± 4.6	0.9 [b] ± 0.1
	G2	96.7 [b] ± 4.1	28.5 [b] ± 1.1	25.6[bc] ± 1.3	60.6[ab] ± 3.8	0.9 [b] ± 0.1
3rd wk	G1	71.7 [e] ± 5.8	23.6 [d] ± 1.1	28.8[ab] ± 1.6	51.9[bcd] ± 4.8	0.9 [b] ± 0.1
	G2	91.4 [c] ± 3.2	28.2 [b] ± 0.9	30.8 [b] ± 0.7	45.8 [de] ± 2.8	0.9 [b] ± 0.1
4th wk	G1	82.1[bc] ± 5.6	24.1 [c] ± 0.4	30.7 [b] ± 0.8	43.3 [de] ± 2.4	0.9 [b] ± 0.1
	G2	81.1[bc] ± 3.3	24.4 [c] ± 1.1	23.9 [d] ± 0.8	40.3 [e] ± 1.9	0.8 [b] ± 0.1
P value		0.002	0.001	0.001	0.458	0.555

G1: Control group G2: Excede

Means carrying different superscripts in the same column are significantly different at (P ≤ 0.05) or highly significantly different at (P < 0.01).

Means carrying the same superscripts in the same column are non-significantly different at (P >0.05).

Bacteriological examination of the collected swabs revealed a highly significant increase (P < 0.01) in log Total *Pasteurella* count (log TPC) of the treated group (G2) when compared to the control group (G1). On the other hand; a non-significant differences was revealed in log Total Bacterial Count (log TBC) and log Total Enterobacteriaceae count (log TEC) of the treated group (G2) when compared to the control group (G1). The highly significant increase (P < 0.01) in log Total *Pasteurella* count (log TPC) was clear at P1; P2 and P3 in the treated group **(Table 4)**.

Table 4:-Log Total Bacterial count (log TBC), Log Total *Pasturella* count (Log TPC) and Log Total Enterobacteriaceae count (Log TEC) in nasal swabs from Excede injected and control animals.

Log bacterial count / ***Treatment***		***Log Total Bacterial Count***	***Log Total Pasteurella Count***	***Log Total Enterobacteriacae Count***
Grs	**G1**	$3.8^{ab} \pm 0.1$	$1.9^{b} \pm 0.2$	$0.39^{a} \pm 0.1$
	G2	$4.1^{a} \pm 0.1$	$2.7^{a} \pm 0.2$	$0.26^{a} \pm 0.1$
P value		0.013	0.001	0.026
Zero	**G1**	$3.96^{ab} \pm 0.36$	$2.17^{ab} \pm 0.43$	$1.14^{a} \pm 0.49$
	G2	$4.04^{ab} \pm 0.13$	$1.92^{ab} \pm 0.23$	$0.14^{bc} \pm 0.14$
1st wk	**G1**	$3.80^{ab} \pm 0.35$	$2.31^{c} \pm 0.45$	$0.29^{bc} \pm 0.19$
	G2	$4.45^{a} \pm 0.19$	$3.06^{a} \pm 0.26$	$0.18^{bc} \pm 0.12$
2nd wk	**G1**	$4.24^{b} \pm 0.22$	$1.02^{d} \pm 0.49$	$0.56^{b} \pm 0.38$
	G2	$4.24^{b} \pm 0.15$	$3.12^{a} \pm 0.15$	$0.18^{bc} \pm 0.12$
3rd wk	**G1**	$3.06^{c} \pm 0.50$	$1.48^{d} \pm 0.47$	$0.00^{c} \pm 0.00$
	G2	$4.07^{ab} \pm 0.22$	$2.89^{b} \pm 0.25$	$0.44^{abc} \pm 0.19$
4th wk	**G1**	$3.9^{ab} \pm 0.24$	$2.49^{ab} \pm 0.32$	$0.00^{c} \pm 0.00$
	G2	$3.58^{ab} \pm 0.19$	$2.32^{ab} \pm 0.31$	$0.38^{abc} \pm 0.17$
P value		0.089	0.011	0.009

G1: Control group G2: Excede

Means carrying different superscripts in the same column are significantly different at ($P \leq 0.05$) or highly significantly different at ($P < 0.01$).

Means carrying the same superscripts in the same column are non-significantly different at ($P > 0.05$).

Temperature revealed a significant ($P \leq 0.05$) strong negative correlation (-0.707) with WBCs count. On the other hand relative humidity revealed a significant ($P \leq 0.05$) intermediate negative correlation (-0.647) with hemoglobin concentration; a significant ($P \leq 0.05$)strong negative correlation (-0.721) with log TEC; a highly significant ($P < 0.01$) strong negative correlations (-0.777; -0.807) with MCHC and WBCs count; respectively **(Table 5a)**.

Table 5.a:-Ambient temperature correlations (Above Diagonal) and Relative Humidity correlations (Below diagonal) with Log bacterial counts and hematological parameters.

	Temp	***Log TBC***	***Log TPC***	***Log TEC***	***Hb***	***RBCs***	***MCHC***	***WBCs***	***Platelets***
RH	**1**	0.340	0.580	-0.448	-0.376	0.371	-0.482	-0.707*	-0.037
log TBC	0.005	**1**	0.419	0.198	0.156	0.400	0.156	0.076	-0.013
log TPC	0.483	0.419	**1**	-0.145	-0.538	0.540	0.063	-0.624	-0.367
log TEC	-0.721*	0.198	-0.145	**1**	0.455	0.267	0.857**	0.703**	0.210
Hb	-0.647*	0.156	-0.538	0.455	**1**	0.056	0.421	0.826**	0.257
RBCs	-0.071	0.400	0.540	0.267	0.056	**1**	0.456	-0.147	0.065
MCHC	-0.777**	0.156	0.063	0.857**	0.421	0.456	**1**	0.559	0.039
WBCs	-0.807**	0.076	-0.624	0.703*	0.826**	-0.147	0.559	**1**	0.399
Platelets	-0.054	-0.013	-0.367	0.210	0.257	0.065	0.039	0.399	**1**

***. Correlation is highly significant ($P < 0.01$).*

**. Correlation is significant ($P < 0.05$).*

^{NS}. Correlation is non-significant ($P > 0.05$).

r = 0.1 – 0.39 represent weak correlation; r = 0.40 – 0.69 represent intermediate correlation; r = 0.70 – 1.00 represent strong correlation.

Temperature revealed a significant ($P < 0.05$) strong positive correlation (0.734) with Albumin; a highly significant ($P < 0.01$) strong positive correlation (0.823) with ALT and a significant ($P < 0.05$) intermediate negative correlation (-0.690) with Creatinine. Relative humidity revealed a significant ($P < 0.05$) intermediate positive correlation (0.686) with Albumin (Alb); a significant ($P < 0.05$) strong positive correlation (0.746) with ALT and a significant ($P < 0.05$) intermediate negative correlation (-0.695) with Creatinine **(Table 5b)**.

Table 5.b:-Ambient temperature correlations (Above Diagonal) and Relative Humidity correlations (Below diagonal) with the biochemical parameters.

	Temp	*TP*	*Alb*	*Glob*	*TG*	*TC*	*Gluco*	*ALT*	*AST*	*Urea*	*Creat*
RH	**1**	0.052	0.734*	-0.622	0.471	0.169	0.614	0.823**	-0.507	-0.090	-0.690*
TP	0.104	**1**	0.008	0.472	-0.385	0.051	-0.029	-0.075	-0.154	0.074	-0.072
Alb	0.686*	0.008	**1**	-0.878**	0.659*	0.354	0.573	0.833**	-0.684*	-0.470	-0.593
Glob	-0.555	0.472	-0.878**	**1**	-0.765**	-0.287	-0.519	-0.770**	0.529	0.449	0.488
TG	0.332	-0.385	0.659*	-0.765**	**1**	0.349	0.155	0.367	-0.230	-0.521	-0.487
TC	0.429	0.051	0.354	-0.287	0.349	**1**	0.128	0.120	-0.469	-0.525	-0.100
Gluco	0.503	-0.029	0.573	-0.519	0.155	0.128	**1**	0.646*	-0.568	-0.227	-0.148
ALT	0.746*	-0.075	0.833**	-0.770**	0.367	0.120	0.646*	**1**	-0.577	-0.028	-0.708*
AST	-0.419	-0.154	-0.684*	0.529	-0.230	-0.469	-0.568	0.577	**1**	0.218	0.170
Urea	-0.360	0.074	-0.470	0.449	-0.521	-0.525	-0.227	-0.028	0.218	**1**	0.017
Creat	-0.695*	-0.072	-0.593	0.488	-0.487	-0.100	-0.148	-0.708*	0.170	0.017	**1**

***. Correlation is highly significant (P < 0.01).*
**. Correlation is significant (P < 0.05).*
^{NS}. Correlation is non-significant (P >0.05).
r = 0.1 – 0.39 represent weak correlation; r = 0.40 – 0.69 represent intermediate correlation; r = 0.70 – 1.00 represent strong correlation.

Discussion:-

The physical component of the calf's environment included the space available and the surfaces with which the animal comes into contact with. Calves placed in group pens should be provided with enough feeder space to allow all calves access. Water availability should also provide easy access, especially to the small, young calf. Another environmental stressor of the calf's environment which may have a greater impact on health and well-being is the waste management system **(Webster, 1983).** Elevated ammonia levels, can cause damage to the lung epithelium and participate respiratory disease. The calf may be continually exposed to these gases with the accumulation of manure and urine. Our observations during the regular farm visits; revealed a lack in the floor space of calves' hutch; signs of malnutrition were clear on large number of calves; the usage of dirty floor system contributed the accumulation of urine which in turn caused the raised levels of ammonia. Ammonia high levels were washed by the action of strong air movement; although its effect wasn't clearly investigated during this study.

Cold and fluctuating air temperatures and excessive wind and moisture are common weather-related thermal (cold) stressors during this period and may contribute to reduced survival of newborn calves. Environmental stresses were closely related to the development of pneumonia manifestation among the injected animals.

The relative increase in albumin level may be attributed to the decreased globulin level which is synchronized with the observed decrease in the total leukocytes count in treated animals and this may be attributed to suppression of the primary immune defense by cortisol released as a response to stress resulted from injection of drug where stress is well known to alter the leukocyte count on their distribution **(Suresh and Koner, 2012).**

The increased glucose level in the injected calves after 1 week from injection can be explained by release of cortisol from the adrenal cortex as a major stress alterations which resulted from injection of the drug **(Mudron et al., 2005)** furthermore, the elevated level of ALT activity in treated animals may be allied to possible hepatocellular damage caused by drug administration **(Sivaramakrishnan et al., 2008)**
As it's known *pasteurella* is a normal inhabitant of the upper respiratory tract in normal unstressed animals and does not usually cause disease, however when animals are exposed to various stress factors as handling and ambient temperature fluctuations they become more susceptible to growth of the organism in the lower respiratory tract, resulting in severe pneumonia as stress may increase secretion of cortisol which in turn alter immune functions and increase susceptibility to bacterial pneumonia **(Filion et al., 1984)** and this can explain the increased Log TPC in injected calves.

Conclusion & Recommendation:-

The present study revealed that morbidity rate although of Excede (Ceftiofur) injection was high among dairy calves. Excede (Ceftiofur) failed to produce promising protective and preventive effects in dairy calves. On the

contrary; Excede injection present a stress factor on the animals; which in turn collaborate with other overwhelming challenges as cold weather; bad management; poor nutrition and poor housing system that contribute the falling down of immune system.

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