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Blood Parameters and Immuno-Response of Dam Friesian Cows and Immunity of Their Newborn Treated with L-carnitine

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THIRTY healthy Friesian cows that were chosen in the late pregnancy period for this study. Three groups of ten cows each were formed from the experimental cows. Cows in 1st without L-carnitine supplement served as a control group (G1), and supplemented with L-carnitine supplementation 2 and 4g/cow/day in the 2nd (G2) and 3rd group (G3), respectively from 20 days pre-calving until 3 days post-calving. A significant improvement of L-carnitine supplemented diets on counts of red (RBC's) and white blood cells (WBC's) and concentration of hemoglobin (Hb) in cows. The haematological values of treated cows were greater than those of the control group. The immunological response of the treated cows was greatly enhanced by the L-carnitine treatment. Treated cows were significantly highest lymphocytes in G3 and reduced monocytes than in G1. The levels of total proteins, triglyceride and HDL (high-density lipoprotein) in blood plasma were higher in G3 than in G2 and G1. However, cholesterol, lipoprotein, and LDL (low-density lipoprotein) concentrations were significantly decreased in G3 and G2 than in G1. Immunoglobulins IgG, IgA and IgM concentrations on different days of cows were significantly higher in G2 and G3 than in G1 in newborn calves.

Treatment with L-carnitine level of 4g/head/day improved the immune response of treated cows. As well as L-carnitine improved the IgG and IgA, IgM and total Ig concentrations in plasma of newborn calves with high levels of L-carnitine.

Keywords: Friesian cows, L-carnitine, Blood parameters, Newborn, Immunity.

Introduction

L-carnitine, a naturally occurring important metabolite, has increased bioactivity. L-carnitine is required for transporting activated fatty acids into the mitochondria, where they are converted to ATP by -oxidation. Carnitinepalmitoyltransferase I transports activated fatty acids into mitochondria, which exchanges carnitine for CoA to generate L-carnitine, which is the rate-limiting step in -oxidation [1].

L-carnitine is an essential amino acid that is produced in some organs (liver and kidneys) to lysine and methionine. L-carnitine is essential for the synthesis of energy in cells via mitochondrial – oxidation [2]. Carnitine is involved in a number of metabolic processes, including the oxidation of long-chain fatty acids, ketosis regulation, immune system support, antioxidant system enhancement, and reproductive improvement [3].L-carnitine enhanced erythrocytes (RBC) counts and packed cell volume (PCV) levels, L-carnitine improves its effect on erythropoiesis, erythrocyte stability, and hyperlipidemia [4].

The amino acid L-carnitine has been shown to improve cardiac function by increasing fatty acid transport into mitochondria and immunomodulation [5].

Immunoglobulin concentration in colostrums of Holstein cows was observed to range from 2.72 to 8.85 g/dL [6]. While, immunoglobulin G (IgG) concentration in bovine colostrums collected during

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the first milking of Holstein cows was 1.54 g/dL [7]. Colostrums receive immunoglobulin G1 from blood [8]. IgG1 and IgG2 are the two subtypes of bovine IgG, respectively [9].

The aim of this study was to invistigatethe effects of different levels of L-carnitine supplementation on immunoglobulin concentration, some blood parameters of Holstein cows and newborn calving during 1 to 3 days post-calving.

Materials and Methods

The current study was conducted between October 2020 and June 2021 at the Sakha Animal Production Research Station, Animal Production Research Institute, Egypt. The Animal Care and Ethics Committee of Kafrelsheikh University in Egypt granted permission for this study to be carried out (license number: KFS1345/10).

Animals and management:

In this study we used 30 healthy Friesian cows with average live body weight (BW) of 600±22.5 kg, ages 43 to 58 months, and 2-3 parities. All cows were selected to be during the late period of pregnancy (20 days pre-calving).

System of feeding:

The experimental Friesian cows were fed concentrate feed mixture (CFM), corn silage, and rice straw dietary, as recommended by NRC [10] for dairy cows based on their milk yield and LBW. The CFM had 28% yellow maize grains, 35% undecorticated cotton seed cake, 32% wheat bran, 3% molasses, 1% limestone, and 1% common salt. According to A.O.A.C., the chemical analysis of feeds on a DM basis was determined [11].

Before the start of the study cows were fed a basal ration consisting of 40% CFM, 26% corn silage (CS and 34% rice straw (RS pre-partum and 55% CFM, 30% CS and 15% RS post-partum. The experimental cows were separated into three similar groups with ten cows in each. Cows without L-carnitine supplement in 1st served as a control group (G1), and supplemented with L-carnitine supplementation 2 and 4 g/cow/day in the 2nd (G2) and 3rd (G3), respectively, from 20 days pre-calving to 3 days post-calving.

Experimental procedures:

Blood sampling:

Blood samples of all cows in each group were drawn from the jugular vein in tubes with anticoagulant twice a week throughout the experiment. The obtained blood samples were centrifuged at 4000 rpm for 10 min to extract the blood plasma, and then stored frozen at -20 °C for chemical testing. Using a completely digital haematology counter (Laboratories, USA), haematological parameters such as red blood cells (RBC's) and white blood cells (WBC's) counts, packed cell volume (PCV, %), and haemoglobin concentration were counted or measured in fresh whole blood. Using commercially available kits, the levels of total proteins, lipoprotein, LDL cholesterol, glucose, triglycerides, HDL, urea-N, and AST (aspartate aminotransferase) and ALT (alanine transaminase) activities in the plasma were measured.

Bovine IgG (ELISA kits) were used to measure the immune-globulins (IgG) levels in blood plasma in accordance with the instructions provided by the kit's respective manufacturer (Alpha Diagnostic International, Texas, USA, and Kamiya Biomedical Company, Seattle, Washington, USA). According to the instructions in the operating manuals, the levels of plasma -hydroxybutyric acid (enzymatic-rate technique; Randox Clinical Diagnostic Company) and Non-Esterified Fatty Acids (NEFA) (enzymatic method; Yulan Biotechnology Research Institute) were assessed [12].

Statistical analysis:

The SAS [13] was used to do a statistical analysis for the collected data. Duncan's Multiple Range Test was used to see whether there were significant changes at 0.05 between treatment groups [14]. The statistical model was:

$$Y_{ij} = \mu + T_i + e_{ij}.$$

Where:

Yij = the Observed traits

μ = Means

Ti = Groups of experimental (1= G1, 2=G2 and 3=G3)

eij = Error

Statistical analyses of immunoglobulin concentrations in blood were a factorial design. The following models were used to statistical analyses of immunoglobulin concentrations in blood model was:

$$Yijk = Hi + Sj + HSij + eijk$$

Where Yijk is the examined variable dependency, eijk is the random residual effect, Hi is treatment influence (I 1 & 2), Sj is day effect (j, 1, 2, & 3), HSij is treatment effect and interaction of day, and eijk is the overall mean.

Results and discussion

Haematological parameters and immune response:

Results in Table (1) presents a significant ($P<0.05$) improvement of L-carnitine supplemented diets on RBC's and WBC's counts and Hb concentration of cows. Higher haematological indicators were present in treated cows compared to the control group. However, percentages of PCV were not affected by L-carnitine supplementation. RBC and WBC counts as well as the proportion of lymphocytes rise by 8.3, 13.52, and 4.02%, respectively, in G3 compared to G1. With G1, there was a 10.85% increase in Hb concentration. Improvements in immune system responsiveness may be the cause of the observed improvements in

haematological characteristics in cows fed L-carnitine.

The immune response of the treated cows was greatly ($P<0.05$) improved by the L-carnitine supplementation. In comparison to the control group, treated cows had significantly ($P<0.05$) lower monocytes and significantly ($P<0.05$) higher lymphocytes in G3 and G2. However, percentages of granulocytes were not affected by L-carnitine supplementation. L-carnitine is which plays improvements the immunity response of the cow. L-carnitine supplementation improves immunity, health, and performance [15].

TABLE 1. Haematological parameters and immune response as affected by L-carnitine

Items	Treatment groups		
	Control	G2	G3
Haematological parameters			
RBCs (10^6/mm^3)	3.85±0.09^{b}	4.05±0.11^{ab}	4.17±0.08^{a}
PCV%	35.94±1.2	37.40±0.97	36.92±1.2
HP (g/dl)	10.69±0.26^{b}	11.22±0.28^{ab}	11.85±0.26^{a}
WBCs (10^6/mm^3)	6.14±0.23^{b}	6.59±0.30^{ab}	6.97±0.22^{a}
Immune response			
Lymphocytes (%)	56.77±0.52^{b}	58.83±0.53^{a}	59.05±0.64^{a}
Neutrophils (%)	37.66±0.50	36.66±0.53	36.38±0.51
Monocytes (%)	4.33±0.31^{a}	3.88±0.33^{ab}	3.55±0.32^{b}
Eosinophils (%)	0.94±0.27	0.61±0.23	0.77±0.17
Basophils (%)	0.27±0.27	0.16±0.09	0.22±0.12

G2 and G3: Cows supplemented with L-carnitine 2 and 4g/cow/day, respectively.

a and b Within the same row, group means represented by the same superscripts are not significantly different at ($P>0.05$)**.**

The results agree with Karadeniz *et al*. [16] found that broilers RBC and haemoglobin significantly improved when L-carnitine was introduced to their diets. Also, Moretti *et al*. [17] found that there was a very increased in the number of lymphocytes in treated groups when compared to control group, since L-carnitine at high concentrations delayed the death of lymphocytes immune cells. Also, Soliman *et al*. [18] in broilers, oral administration of L-carnitine resulted in an increase in RBC count and Hb concentration. Chicks fed food supplemented with L-carnitine had an increased RBC count as well as Hb concentration than those fed with control diet [19].

Awad *et al*. [20] found that ducklings fed diets supplemented with L-carnitine had considerably higher lymphocyte cell percentages than those fed a control diet. In addition to the oxygen provided by RBCs, are required and must be adapted to the current energy needs. Within the first week after calving, carnitine synthase genes in bovine hepatocytes and uptake of L-carnitine was found to be up-regulated and may point to a key period for energy metabolism. By moving activated long-chain fatty acids through the inner mitochondrial membrane and into the mitochondrial matrix, where -oxidation takes place, L-carnitine contributes significantly to the energy production cycle in the mitochondria [21].

Acetyl coenzyme regenerates in part thanks to L-carnitine. It can protect antioxidant enzymes from additional peroxidative harm [22]. In vitro and in vivo tests, L-carnitine has also been proven to stabilize membranes and prevent lipid oxidation in RBCs [4, 23]. Because the supply of antioxidants decreases during parturition and breast feeding due to decreasing feed intake, a dietary supplement of L-carnitine may aid to ameliorate the oxidative state [24, 25], with probable implications for haematological characteristics.

Carnitine works by interacting with fatty acids, and this method may have an impact on cell function. Carnitine impacts the metabolism of arachidonic acid and, as a result, blood platelet activities by Pignatelli *et al*. [26]. Arachidonic acid is essential for blood platelet activation. Platelet activation and oxidative stress are both influenced by carnitine's interference with arachidonic acid metabolism. Arachidonic acid and collagen induce platelet superoxide anion production, which is inhibited by carnitine [26].

Carnitine regulates blood platelet activation [26]. Platelet activation involves a number of biological reactions, including adhesion, aggregation, and secretion of chemicals contained in platelet granules [27, 28]. Platelet activation is a complicated process that aids in hemostasis, as well as is implicated in inflammatory and cancerous processes [29, 30].

Biochemical parameters of blood

Results in Table (2) reported that the concentrations of protein (TP), triglyceride, and HDL in blood plasma of calves were significantly ($P<0.01$) higher in G3 than in G2 and control (G1). However, lipoprotein, cholesterol, and LDL concentrations were significantly lower ($P<0.01$) in G3 and G2 than in G1. While, both the AST and ALT activity were quite similar. The current plasma total protein values are within the normal range and well-concordant with those found by other researchers in calf studies [31].

TABLE 2. Biochemical parameters concentration in plasma as affected by L-carnitine

Items	Treatment groups		
	Control	G2	G3
Proteins (g/dl)	6.40± 0.05[b]	6.63 ± 0.03[b]	8.08±0.14[a]
Lipoprotein (??)	0.79± 0.011[a]	0.66± 0.003[b]	0.62± 0.019[b]
Cholesterol (mg/dl)	139.0±1.57[a]	129.44±1.65[b]	124.33±1.33[c]
High density lipoprotein (HDL, mg/dL)	72.00± 0.28[c]	80.77±1.11[b]	84.33± 0.33[a]
Low density lipoprotein (HDL, mg/dL)	37.00± 0.270[a]	33.66± 0.68[b]	21.0± 0.28[c]
Triglyceride (mg/dl)	91.33±0.60[b]	87.88±1.53[ab]	86.00± 0.68[a]
AST (U/L)	42.11± 1.79	43.22 ±0.98	46.33 ±0.33
ALT (U/L)	24.66± 0.44	25.44 ±0.58	24.88 ±0.96

G2 and G3: Cows supplemented with L-carnitine 2 and 4g/cow/day, respectively.

[a, b and c] Within the same row, group means identified by the same superscripts do not differ significantly (P <0.01).

Carlson *et al.* [32] reported that carnitine administration lowered triglyceride level in the liver while simultaneously increased the concentration of glycogen. In addition, Massy *et al*. [33] and Naini *et al*. [34] found that L-carnitine decreased total plasma cholesterol and triglyceride levels. L-carnitine supplementation has been linked to considerable Lipoprotein reduction [35]. The effect of L-carnitine on lipoprotein level is not totally known. However, the cause for these effects could be that L-carnitine reduces liver production of Lipoprotein by increasing fatty acid breakdown in mitochondria [36].

Results of this study agree with Carlson *et al*. [32] who found that carnitine supplementation lowered total lipid and triglyceride contents in the liver while simultaneously enhancing glycogen concentration. Also, Massy *et al*. [33] and Naini *et al*. [34] found that levels of cholesterol and triglyceride decreased by L-carnitine. Also, Citil *et al*. [3] found that supplementing ewes diets with 500 mg carnitine resulted in lower serum triglyceride, urea, and cholesterol levels.

Recently, Yousefinejad *et al*. [37] found that HDL level was greatest in the L-carnitine group. Foroozandeh *et al*. [38]; Serban *et al*. [35]; Yousefinejad et al. [39] found that L-carnitine reduced both cholesterol and LDL levels in blood.

Immune-globulins concentrations in cows

Results in Table (3) shows that plasma immunoglobulins IgG, IgA, and IgM concentrations on different days for cows were not differ significant in all groups. The IgG and IgA levels in the plasma of cows in G2 and G3 decreased after calving up to 3 days as compared to G1. L-carnitine supplemented was not significant affect on the immune-globulins concentrations plasma of cows**.**

In the same line, Newton and Burtle [40] observed that L-carnitine supplementation during pregnancy had no effect on plasma IgG concentration in cattle, while Uhlenbruck [41] found that L-carnitine supplementation during pregnancy has no effect on plasma IgG concentration in cattle. Also, Kacar *et al*. [42] 2008) discovered that on the first and third days after delivery, plasma IgG concentrations of cattle given L-carnitine and control cattle were not substantially different.

Immune-globulins concentrations in newborn calves

The concentration of IgG and IgA were significantly (P<0.01) increased with the treatment of L-carnitine in G2 and G3 comported to the control group. This increase was about 6.13 and 8.68% of IgG and 67.7 and 75.8% of IgA on overall means, respectively. However, the IgM concentration was significantly (P<0.01) higher in G3 than in G1. There were no significant differences between G3 and G2 (Table 4). After birth and for three days after calves were born, IgG concentration rise in all groups. In addition, from birth until the third day in both groups, plasma immunoglobulin concentrations rise gradually with age.

The IgG, IgA, and IgM concentrations in the plasma of cows and calves were higher significantly by L-carnitine (P<0.01). The latest findings are in line with previous findings those of [43], who demonstrated that L-carnitine supplementation improved blood IgG concentration in broilers (P<0.05). In broilers [44] found that L-carnitine treatment significantly improved IgG concentration.

Thangasamy *et al*. [45] reported that L-carnitine increased concentrations of IgG and IgA in a significant manner in aged animals. The calves acquire a high concentration of L-carnitine via milk, which could be one mechanism by which L-carnitine improves the IgG and IgA concentrations in their blood. During pregnancy and lactation, supplementing cows with L-carnitine resulted in a moderate increase in L-carnitine concentrations in blood and milk.

TABLE 3. Concentration of immune-globulins (Ig, mg/ml) in the blood plasma of cows in the experimental groups.

Items	Day	Treatment groups			Overall means
		Control	G2	G3	
IgG	1	26.00±0.84	27.96±0.73	28.00±0.94	27.32±0.95
	2	25.16±0.73	26.33±0.60	26.66±0.83	26.05±0.82
	3	24.63±0.83	25.23±0.54	25.02± 0.57	24.96±0.67
	Overall means	25.36±0.74	26.51±0.62	26.56±0.58	
IgA	1	3.35±0.11	4.02±0.09	4.22±0.12	3.86±0.11
	2	2.67±0.36	3.26±0.22	3.96±0.28	3.30±0.27
	3	3.06±0.08	3.58±0.17	3.87±0.06	3.50±0.12
	Overall means	3.03±0.21	3.62±0.19	4.02±0.29	
IgM	1	7.05±0.17	8.06±0.28	8.90±0.15	8.00±0.21
	2	6.23±0.38	7.04±0.25	7.30±0.17	6.86±0.34
	3	6.58±0.22	8.05±0.13	7.23±0.14	7.29±0.21
	Overall means	6.62±0.35	7.72±0.42	7.81±0.27	

G2 and G3: Cows supplemented with L-carnitine 2 and 4g/cow/day, respectively

TABLE 4. Total immune-globulin concentration (Ig, mg/ml) in the blood plasma of experimental group calves

Items	Day	Experimental groups			Overall means
		Control	G2	G3	
IgG	1	16.83±0.12	17.46±0.66	18.06±0.14	17.45 ± 0.52^{C}
	2	18.96±0.03	19.73±0.16	20.56±0.14	19.75 ± 0.45^{B}
	3	20.53±0.29	22.56±0.13	22.56±0.24	21.88 ± 0.53^{A}
	Overall means	18.77 ± 0.24^{b}	19.92 ± 0.15^{a}	20.39 ± 0.16^{a}	
IgA	1	1.43±0.24	2.63±0.03	2.75 ±0.06	2.27 ± 0.09^{C}
	2	2.16±0.12	2.94±0.03	3.11±0.04	2.73 ± 0.08^{B}
	3	1.99±0.05	3.79±0.04	3.94±0.05	3.24 ± 0.07^{A}
	Overall means	1.86 ± 0.07^{b}	3.12 ± 0.04^{a}	3.27 ± 0.06^{a}	
IgM	1	2.37±0.18	3.62±0.13	3.43±0.03	3.14 ± 0.12^{B}
	2	3.33±0.66	4.66±0.28	4.10±0.10	4.03 ± 0.11^{A}
	3	2.96±0.57	3.53±0.03	4.43±0.12	3.64 ± 0.12^{B}
	Overall means	2.89 ± 0.35^{b}	3.94 ± 0.17^{ab}	3.99 ± 0.18^{a}	

G2 and G3: Cows supplemented with L-carnitine 2 and 4g/cow/day, respectively.

a and b Within the same row, group means represented by the same superscripts are not significantly different at (P>0.01).

Tian *et al*. [46] stated that total IgA and IgG of antigen-specific responses have been shown to be immunomodulated by dietary L-carnitine intake. A long-lasting increased IgG response induced by L-carnitine treatment could be crucial for improving protective immunity following vaccination. From this result, cows receiving increased L-carnitine via milk may have increased Ig of antigen-specific responses, which could lead to increased immunoglobulin levels in calves.

On the days 2, 10, and 20 of lactation, [47] discovered that adding CLA to the diet enhanced blood IgG levels in sows and piglets (P 0.05). Furthermore, Rossi *et al*. [48] found that born from sows fed carnitine diets from seven days before parturition had increased serum IgG concentrations on day 21 postpartum (P<0.05). Kacar *et al*. [42] found Injections of L-carnitine into pregnant cows had no effect on IgG, GGT, protein, or albumin concentration in the cows or their newborn calves.

Conclusion

This study demonstrated that the treatment during Pre- and Post-partum with L-carnitine at the level of 4g/head/day improved the immune response of treated cows. As well as improve biochemical parameters of blood in Friesian cows. Also, with high level of L-carnitine improved the IgG and IgA, IgM and total Ig concentrations in plasma of newborn calves.

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The authors declare that the present study has no financial issues to disclose.

Conflict of Interest

None

Author`s contributions

All authors contributed to the study's conception, and design. Data collection, examination and experimental study were performed by ERS, AMM, YME and MAA. All biochemical analysis and data analysis were performed by MAA, ERS and ZRG. AMM, AAB, AMM and YME drafted and corrected the manuscript; MAA and YME revised the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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صفات الدم والاستجابة المناعية لأمهات الأبقار الفريزيان والمناعة في العجول حديثة الولادة المعاملة بال-كارنيتين

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تهدف هذه الدراسة إلى معرفة تأثير L-Carnitine على الاستجابة المناعية في أبقار الفريزيان وكذلك مستوي المناعة قي العجول الحديثة الولادة.واستخدم في هذه الدراسة ثلاثون بقرة فريزيان في المرحلة الأخيرة من الحمل وقسمت إلى ثلاث مجموعات متماثلة (10 في كل مجموعة) المجموعة الأولى (G1) مجموعة مقارنة بدون أي إضافة، بينما تم إضافة 2 و4 جم ل-كارنتين/ بقرة / يوم في المجموعة الثانية (G2) والثالثة (G3) على التوالي خلال الفترة التجريبية من اليوم 20 قبل الولادة حني اليوم الثالث بعد الولادة.

وأظهرت النتائج أن إضافة ل-كارنتين ادت الى تحسن فى عدد كرات الدم الحمراء والبيضاء وتركيز الهيموجلوبين في الدم للأبقار المعاملة. كما أدت المعاملة بالكارنتين إلي تحسين الاستجابة المناعية للأبقار. ارتفاع تركيز كل من البروتينات الكلية والدهون الثلاثية والليبوبروتين عالى الكثافة في بلازما دم الأبقار بشكل ملحوظ في المجموعة الثالثة عنها في المجموعة الثانية و المجموعة الأولي. بينما انخفضت تركيزات الكوليستيرول و الليبوبروتين منخفض الكثافة بشكل ملحوظ في المجموعة الثالثة والمجموعة الثانية مقارنة بالمجموعة الأولي. وارتفع تركيز كل من مشتقات المناعة بشكل معنوي في المجموعة الثالثة و المجموعة الثانية مقارنة بالمجموعة الأولي قي دم العجول حديثة الولادة .

ونستنتج من هده الدراسة أهمية إضافة 4جم ل-كارنيتين لكل رأس في اليوم حيث أدت إلي تحسين مستوي الاستجابة المناعية وبعض قياسات الدم فى الابفار وكذلك تخسين مستوي المناعة للعجول الفريزيان حديثة الولادة،

الكلمات الدالة: ابقار الفريزيان وال-كرانتين وخصائص الدم والعجول حديثة الولادة والمناعة.