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Evaluation of Biochemical and Hematological Parameters in Postpartum Holstein Dairy Cows Following Supplementation of Immunofin® Herbal Extract

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Abstract

BACKGROUND: The incidence of diseases among dairy cows is high in the postpartum period, which reduces dairy enterprise and higher demands for antibiotics. Considering this, the notion of promoting heath and reducing antibiotic application in dairy cow medicine by taking advantages of bioactive phytochemicals in herbal extracts is emerging.

OBJECTIVES: This study's main purpose was to investigate the effects of supplementing Holstein dairy cows' ration in the close-up period by Immunofin® polyherbal aqueous extract on the normal physiology, clinical health, and hematological and biochemical parameters of postpartum dairy cows.

METHODS: In this experiment, the herbal extract was supplemented in a close-up period diet for cows in the treatment group (n=10). However, cows in the control group (n=10) received precisely the same ratio as the placebo. The clinical health of cows was assessed, and some of their biochemical and hematological parameters were compared between the two groups.

RESULTS: Cows in the treatment group had a lower incidence of retained fetal membrane and metritis (P=0.01). In addition, cows in the treatment group had a lower concentration of serum nonesterified fatty acid (P<0.001), and a lower number of peripheral blood leukocytes and lymphocytes count among cows in the treatment group (P<0.0001). In contrast, their mean corpuscular hemoglobin and mean corpuscular volume were higher (P<0.0001).

CONCLUSIONS: In conclusion, Immunofin® supplementation in a close-up period had no negative impacts on prepartum cows' clinical health and could desirably alter some of the clinical, biochemical and hematological parameters.

KEYWORDS: Clinical health, Immunofin, Metabolic status, Polyherbal extract, Transition period

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Introduction

The transition period is one of the most challenging stages in the lifetime of dairy cows, as 30 to 50% of high producers may be affected by one or more peripartum diseases. (Leblanc, 2010) Considering the impact of postpartum cow health in achieving maximum production potential and enhancing economic profitability, it is essential to recognize related risk factors of diseases (Stevenson, 2020). Because of reducing dry matter intake and concurrent increasing energy demands and incompetency of the immune system due to physiologic, endocrine changes, metabolic challenges, and energy deficit are the main predisposing factors, and the critical role of metabolic and immune status in maintaining postpartum health has been revealed in recent years (Bicalho, 2016, Jahan *et al*., 2020).

Considering metabolic status, a high concentration of β-hydroxybutyrate (BHBA) and non-esterified fatty acid (NEFA) has been reported to be linked with significantly increased odds of developing postpartum clinical diseases, including retained fetal membrane, postpartum uterine infections (Qu *et al*., 2014; Chebel *et al*., 2021) and also culling (Seifi *et al*., 2011). Moreover, the prevalence of hepatic lipidosis is relatively high (Gonzaez *et al*., 2011), and a modified hepatic enzymes profile has been reported among cows with left displaced abomasum (Song *et al.*, 2020). There are also reports about hematological changes and neutropenia in cows with a retained fetal membrane (Moretti *et al*., 2015), changed complete blood count in cows with metritis (Cui *et al*., 2019), and mastitis (Guan *et al*., 2020). One of the interesting fields of study in recent years is using phytochemicals to enhance a postpartum cow's health, immunity, and metabolic status (Alves *et al*., 2016; Lopreiato *et al*., 2020). Effects of alkaloid compounds in Chinese traditional herbal medicine (Shi *et al*., 2018), herbal derived antioxidant from green tea (Ma *et al*., 2021), and phytobiotic rich herbal extract on improving the metabolic status of dairy cows has been reported before (Hashemzadeh Cigari *et al.*, 2014).

Immunofin® polyherbal extract (Pars Imendaru CO., Iran) is the commercial mixture of purified bioactive phytochemicals obtained from several medicinal plants, including coneflower *(Echinacea purpurea*), rosemary *(Rosmarinus officinalis),* thyme *(Thymus vulgaris)* and yarrow (*Achillea millefolium)*. Based on manufacturer-provided information, the most important bioactive phytochemical ingredients of the extract are alkamides and alkylamides (isobutylamide, N-methylbutylamine, etc.), polysaccharides (Echinacin, arabinogalactan, xylogalactan), glycosides (caffeic acid and its derivates including rosemarinic acid, chicoric acid, echinacoside), and terpenes (carnosic acid, citronellol, humulene, and caryophyllene). Biologic effects of each compound have been discussed in previous papers (Andrade *et al.*, 2018; Bruni *et al.*, 2018; Gedikoğlu *et al*., 2019). In this study, we supplemented Immunofin® to close-up period ration to investigate its effect on clinical health, metabolic status, and hematologic indices of postpartum cows.

Materials and Methods

Animal Selection and Experimental Design

This study was conducted under heat stress conditions (THI>72) during July and August 2020 in a large industrial dairy farm with 2500 milking cows and an average daily milk yield of 39 kg per cow, located in Malayer, Hamedan province, Iran. Based on our inclusion criteria, pregnant cows on days 245 to 252 of their pregnancy were selected at the beginning of the close-up period. Selected cows (n=20) had a parity number of 1 or 2, a body condition score of 3 to 4, a locomotion score of 1 or 2, and they were healthy and free of any abnormal clinical signs. Cows were kept separately in the same open shed stalls during the experiment. Provided stalls had sandy bedding and cooling equipment, including ceiling fans and sprinklers. All cows were fed twice daily with the same diet during close-up (and in the postpartum period) based on TMR ingredients exceeding the nutritional requirements of cows (Table 1). Moreover, selected cows were vaccinated against FMD, brucellosis, blackleg, and lumpy skin disease according to the standard vaccination program of dairy herds.

Table 1. Dietary ingredients of the ration used to feed the cows in both groups of the study (Dry matter basis).

Item	Amount
Ingredients (% OF DM)	
Corn silage	31.2
Alfalfa hay	14.1
Wheat straw	8.4
Whole cottonseeds	3.1
Ground barley	14.7
Ground corn	6.9
Extruded soybean	1.4
Canola meal	5.9
Fish meal	1.1
Soybean meal	4.3
Sodium bentonite	0.3
Magnesium oxide	0.1
Calcium carbonate	1.1
Glycoline	0.9
Calcium choloride	0.7
Magnesium solphate	1
Transitional feed supplement[1]	3.5
Mineral and vitamin premix[2]	1.4
Chemical composition (% of DM, unless otherwise noted)	
NEL (Mcal/kg)	1.5
DM (%)	42.3
OM	90.8
CP	13.4
NDF	37.7
ADF	23.6
NFC	35.6

[1]Coposition: 3000 ppm of Niacin/kg, 15000 ppm of Choline/kg, 5000 ppm of Chorome/kg.

[2]Coposition:290000 ppm of Ca/kg, 15300 ppm of Mg/kg, 8500 ppm of Mn/kg, 11250 ppm of Zn/kg, 3200 ppm of Cu/kg, 190 ppm of I/kg, 105 ppm of Co/kg, 10000 IU of vitamin A/kg, 250000 IU of vitamin D/kg, 1200000 IU of vitamin E/kg.

Selected cows were randomly allocated to the Immunofin® treatment group (n=10) or served as the control (n=10). Cows in the treatment group received daily 150 mL of pure extract of Immunofin® (Pars Imendaru CO. Iran) in their diet for 21 days of the close-up period. In order to reach enough volume for mixing in the ration, supplementation was done by diluting 1.5 L of Immunofin® with the same volume of drinking water in 1:1 ratio and then pouring of prepared solution into the cows' TMR diet, mixing it manually immediately at the time of feeding. Cows in the control group received exactly the same ratio as the placebo.

Clinical Evaluation

The clinical part of the study was done by comparing the incidence of postpartum uterine disease (retained fetal membrane and metritis) and assessing general health and the amount of dry matter intake (DMI) in the close-up period between study groups. The average dry matter intake (DMI) of cows in each group was calculated by analyzing and subtracting the dry matter content of leftover feed in the feed bank from the dry matter content of provided feed per cow daily. Rumen scoring was done daily, based on 1-5 scale and assessment of rumination was done three times a day, two hours after feeding. Moreover, consistency and digestion scoring of the cows' manure was assessed daily according to 1-5 scale scoring system.

Blood sampling and Hematology

Blood sampling from the jugular vein was done using plain, and EDTA contained venoject tubes. Blood samples were collected from each cow at the beginning time of the study (week -3 or 3 weeks before calving), a week before calving (Week -1), and the first (Week +1), second (Week +2), and third week (Week +3) postpartum. Hematological indices, including the number of white and red blood cells per microliter (μL), hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean

corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), were evaluated. In order to perform the assessment, 10 µL of blood taken from EDTA venoject tubes was analyzed by a fully automated hematology analyzer device, Celltac alpha, model MEK-6550 (NIHON KOHDEN®, Tokyo, Japan), according to the manufacturer's recommended procedure of calibration and applying the special hemolyzing reagent, diluent, and detergent. Differential blood count (Lymphocytes, Monocytes, Eosinophils, Polymorphonuclears, Band cells) and neutrophil to lymphocyte ratio (NLR) were also evaluated by manual microscopy of prepared Giemsa stained (Sigma-Aldrich®) blood smears under oil immersion objective lens 100X magnification.

Serum Biochemistry and Evaluation of the Metabolic Status

Centrifugation of clotted blood was done at 1000 X g at 4ºC for 20 minutes. Separated serum was aspirated from the supernatant by pipette without disturbing the sediment and then transferred to a sterile 10 mL plastic test tube. Samples were stored at –80ºC until the time of analysis. Several biochemical parameters, including the concentration of Non-esterified fatty acids (NEFA), β-hydroxybutyric acid (BHBA), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) in serum, were analyzed by automated biochemistry analyzer (Hitachi 911 Analyzer, Roche diagnostic) according to manufacturer provided reagents and recommended procedures for enzymatic colorimetric method.

Statistical Analyses

Statistical analysis of provided data was done by SAS software (version 9.2, SAS Institute Inc., Cary, NC). Statistical analyses of binary variables were done using the FREQ procedure and Chi-squared test. For analyses of numerical data, first, normality was tested by GLM procedure and Kolmogorov-Smirnov test, and then based on normality test results, TTEST or Mann-Whitney U test was done for comparing two groups. Analyses of related numerical data in different time sessions were done by applying the GLM procedure and repeated measures ANOVA. Significance was declared at P-value< 0.05 and tendency was defined at 0.05 <*P*<0.1.

Results

Clinical Findings

Meticulous clinical observations on a daily basis indicated normal appetite, normal rumen score (score 4), rumination behavior and normal consistency (score 4), and digestibility score (score 3) of cow's manure in the treatment group. Based on these findings, the administered herbal supplement had no adverse effects on the normal digestive physiology of treated cows. No cows with clinical signs of indigestion or other health problems were seen during the study. One of the cows in the control group was deleted from the study because of developing non-responding to downer cow syndrome and being culled following parturition.

The average length of pregnancy (*P*=0.65) and sex ratio of born calves (*P*=0.8) were similar between the two groups. The birth weight of born calves from cows in the treatment group was significantly higher than those born from cows in the control group (40.2±1.05 vs. 39.4±2.42, respectively, *P*=0.03). Cows in the treatment group tended to have a significantly higher yield of colostrum (*P*=0.086) by providing 5.3±0.73 liters of colostrum on average with Brix of 28.6±1.25, in the first postpartum milking session, compared to cows in the control group with 3.55±0.41 liters of colostrum and Brix of 27.6±0.81.

Based on provided data, the average DMI of cows in the treatment group during the close-up period was numerically higher (13.34 ±0.44 vs.12.1±0.6 respectively) than cows in the control group. Still, the difference was not statistically significant (*P*=0.1). Four out of 9 cows (44%) in the control group were affected by the retained fetal membrane and puerperal metritis. In contrast, none of the cows in the treatment group suffered from these postpartum uterine complications (*P*=0.01).

Para-clinical Findings

Serum Biochemistry

The serum concentration of NEFA was higher among cows in the control group compared to cows in the treatment group (*P*=0.033, Figure 1). Regardless of the group, NEFA was higher in the first and second week postpartum than in other time sampling sessions (*P*<0.05). The concentration of BHBA and

ALT was not influenced by the effect of group, time, and interaction of group by time (P> 0.05). However, the concentration of AST was higher in week +1 compared to week -3, week -1, and week +3 (P<0.05) (Table 2).

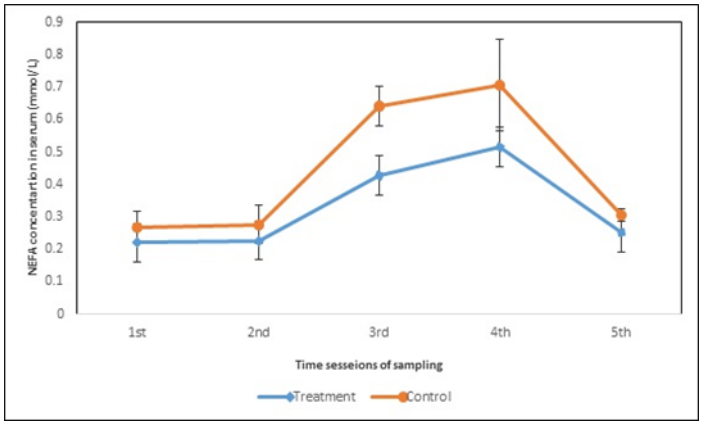

Figure 1. Average nonesterified fatty acid concentration (mmol/L) in the serum of cows in the treatment (n=10) and control (n=9) groups. The serum concentration of NEFA was higher among cows in the treatment group compared to cows in the control group (P=0.033).

Table 2. Mean± SEM of serum concentration of BHBA (mmol/L), ALT (U/L), and AST (U/L) of the cows in the treatment and control group in different time sessions

	Group	Week -3	Week -1	Week +1	Week +3	Week +5
BHBA (Mean ±SEM)	Treatment	0.44±0.07	0.43±0.04	0.71±0.17	0.5±0.07	**0.62±0.08**
	Control	0.58±0.04	0.59±0.07	0.92±0.18	0.61±0.09	**0.76±0.3**
ALT (Mean ±SEM)	Treatment	27.2±1.78	27.4±1.95	25.1±3.43	19.7±1.11	**22.2±2.43**
	Control	25±1.41	25±1.54	23.88±2.64	25.44±1.91	**27.33±0.83**
AST (Mean ±SEM)	Treatment	92.5±6.66^{a}	94.5±6.66^{a}	186.6±35.5^{b}	120.7±9.7^{a,b}	**114±3.07^{a}**
	Control	96.2±9.28^{a}	100.22±9.13^{a}	139.1±28.5^{b}	130.5±8.3^{a,b}	**124.6±4.18^{a}**

Hematology

Compared to the control group, white blood cells (WBC) and blood lymphocytes counts were lesser in the treatment group (P<0.0001, Figure 2 and Table 3). Moreover, WBC and blood lymphocyte counts in both groups were lesser in week +1 than week -1 and +3 (P<0.05). Monocyte count was higher in week -1 than in weeks -3 and +2 (P < 0.05), and eosinophil count was higher in week -1 than in week -3 and week +2 (P=0.011) in both groups. Granulocyte count was not influenced by the effect of group, time, and interaction of group by time (P>0.05). Band cells count was higher in week +2 than weeks -1 and +1, regardless of the group (P<0.05, Table 3). The effect of the group (P=0.19), time (P=0.17), and interaction of group and time (P=0.33) on neutrophils to lymphocytes ratio (NLR) were not significant (Figure 3). Furthermore, mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) was lesser in the control than in the treatment group (P<0.0001, Figures 4 and 5). Regardless of group, MCH was lesser in week -3 and week -1 than in weeks +1, +2, and +3 (P<0.001). Contrary to MCH, red blood cells count, hemoglobin concentration, and hematocrits were higher in week -3 and week -1 than

in week +1, week +2, and week +3 (P<0.01). Moreover, all these three indices were higher in week+1 than week+3 (P<0.05). Mean corpuscular volume (MCV) was higher in week +1 than in other time sessions of the study (P<0.05), and mean corpuscular hemoglobin concentration (MCHC) was lower in week -1 than in week +2 and week +3 (P<0.05), and in week+1 compare to week+3 (P=0.007), irrespective of the group (Table 4).

Table 3. Mean ±SEM of blood lymphocyte, monocyte, granulocyte, band cell, and eosinophil count per uL of the cows' blood in the treatment and control group in different time sessions

	Group	Week -3	Week -1	Week +1	Week +3	Week +5
Lymphocyte (Mean ±SEM)	Treatment	6555.77± 1208.2^{a,b,1}	8517.2± 1764.8^{a,1}	5980.9± 1604.3b,1	8308± 1979.7^{a,b,1}	**8121± 1882.2^{a,1}**
	Control	12700± 2759.5^{a,b,2}	13624.67± 3262.8^{a,2}	11322± 3543.6b,2	12886.78± 3963.9^{a,b,2}	**13709± 4119.1^{a,2}**
Monocyte (Mean ±SEM)	Treatment	292±81.6^{a}	656.3±138.3^{b}	680.8±163.7^{a,b}	137±47a	**454.1±138.9^{a,b}**
	Control	342.33± 73.7^{a}	837.44±284.9^{b}	710.22± 208.1^{a,b}	530.33± 135^{a}	**650.33± 85.4^{a,b}**
Granulocyte (Mean ±SEM)	Treatment	3894.33± 564.6	4426.7±545.6	2981.1±636.2	2952± 675.8	**3334.6± 454.2**
	Control	2888±345.6	4286.77±1739	3335.44±430.5	3516.88± 576.8	**4460.33± 508.5**
Band cell (Mean± SEM)	Treatment	44.3±30^{a,b}	35.6±23.85^{a}	30.3±16.7^{a}	233.1± 116.7^{b}	**119.9± 61.3^{a,b}**
	Control	209.77±103^{a,b}	116.88± 51.89 ^{a}	83.44±45.8^{a}	369.11± 163.6^{b}	**112.66± 45.2^{a,b}**
Eosinophil (Mean± SEM)	Treatment	464.22±158^{a}	154.2±65.5^{a,b}	64.4±29.87^{a,b}	12±12^{b}	**30.4± 23.66^{a,b}**
	Control	382.11± 131.49^{a}	182.44± 66.2^{a,b}	248.88± 126.2^{a,b}	40.55± 22.2^{b}	**365.66± 321.6^{a,b}**

Table 4. Mean ±SEM of Mean corpuscular hemoglobin concentration (g/dl), RBC (10^6/uL), Hematocrit (%) and Hemoglobin (g/dl) of the cows in the treatment and control group in different time sessions.

	Group	Week -3	Week -1	Week +1	Week +3	Week +5
MCHC (Mean ±SEM)	Treatment	36.67±1.08^{a,b}	34.98+0.38^{a}	35.39±0.2^{a,b,1}	36.32±0.19^{b}	**36.71±0.12^{b,2}**
	Control	35.64±0.15^{a,b}	35.14±0.15^{a}	35.46±0.33^{a,b,1}	36.52±0.18^{b}	**37.04±0.39^{b,2}**
RBC (Mean ±SEM)	Treatment	6.48±0.25^{a}	6.31±0.17^{a}	5.99±0.15^{b}	5.46±0.2^{b,c}	**5.28±0.18^{c}**
	Control	6.53±0.22^{a}	6.02±0.16^{a}	5.51±0.15^{b}	5.41±0.2^{b,c}	**5.30±0.18^{c}**
Hematocrit (Mean ±SEM)	Treatment	29.7±0.75^{a}	28.72±0.46^{a}	27.7±0.46^{b}	24.77±0.63^{c}	**23.66±0.73^{c}**
	Control	29.2±0.68^{a}	27.83±1.01^{a}	25.95±0.89^{b}	25.02±0.86^{c}	**24.31±0.61^{c}**
Hemoglobin (Mean ±SEM)	Treatment	10.55±0.25^{a}	10.05±0.17^{a}	9.8±0.16^{b}	8.99±0.21^{b,c}	**8.68±0.25^{c}**
	Control	10.35±0.23^{a}	9.77±0.34^{a}	9.18±0.26^{b}	9.13±0.29^{b,c}	**9±0.22b,2^{c}**

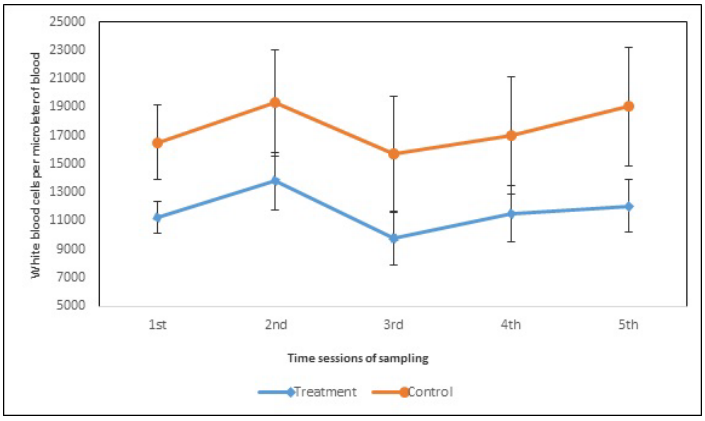

Figure 2. Average white blood cells count per microliter of the blood of cows in treatment and control groups. WBCs count was higher in the control group (P<0.0001).

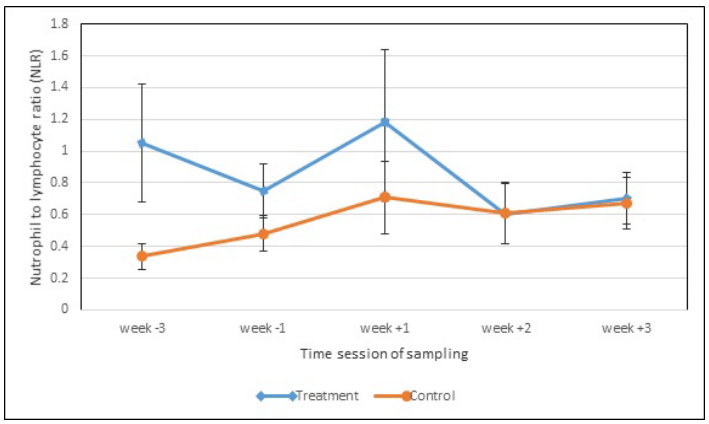

Figure 3. Average neutrophil to lymphocyte ratio. In contrast to the control group, Immunofin® treated cows showed a decreasing pattern of NLR.

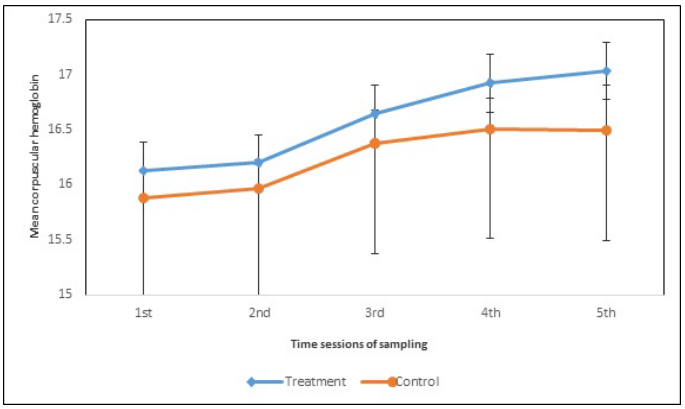

Figure 4. Average Mean corpuscular hemoglobin of cows in treatment and control group. Average MCH was higher in treatment group (P<0.0001).

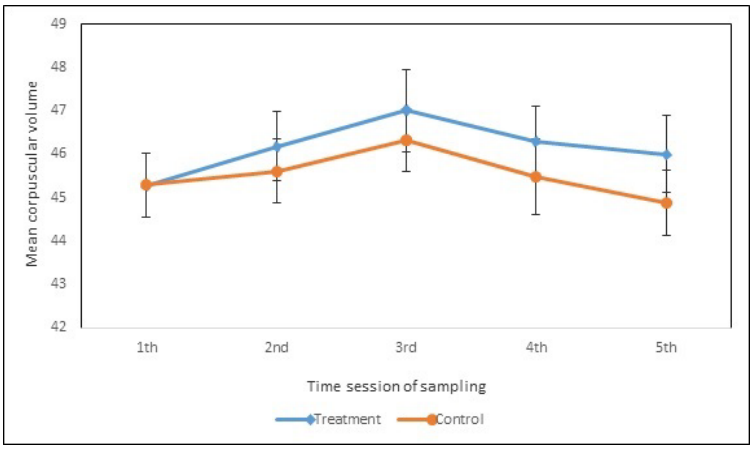

Figure 5. Average Mean corpuscular volume of cows in treatment and control group. Average MCV was higher in the treatment group (P<0.0001).

Discussion

The manufacturer generally recommends Immunofin® for enhancing calf health and reducing the incidence of calfhood diseases. In previous studies, considering the positive effect of some of its ingredients on dairy cows' health, we decided to supplement it as an extra-label herbal medicine to the ration of heat-stressed cows during the close-up period. Heat stress exacerbates metabolic complications and health problems in postpartum dairy cows (Skibiel *et al.*, 2017); therefore, heat stress condition was selected for conducting this study to evaluate the efficacy of Immunofin® in improving metabolic status and health of postpartum cows in the condition which is mostly needed.

The incidence of retention of fetal membrane (RFM) and metritis among cows in the treatment group showed significant reduction. There are several reports about successful usage of herbal medicine in prevention of RFM and metritis (Cui *et al*., 2015; Cui *et al*., 2019). It was reported that prepartum lower amount of DMI together with higher serum concentration of NEFA and proinflammatory cytokines are risk factors of developing RFM and metritis (Qu *et al*., 2014; Dervishi *et al*., 2016). Immunofin effects on DMI and its modulating impact on proinflammatory cytokine could attenuate both of these risk factors and reduce the incidence of RFM.

Cows in the treatment group consumed 1.24 Kgs more dry matter compared to cows in the control group in the close-up period. An increase of DMI could be the consequence of higher palatability of treatment group ration because of pleasant Immunofin® flavor for cows, improved digestibility of consumed ration in rumen caused by polyphenols (Loperiato *et al*., 2014), and stimulatory effect of bioactive alkylamides compounds on appetite, through activation of brain CB1 cannabinoid receptors which trigger orexigenic signals (Kirkham *et al*., 2005; Raduner *et al*., 2006; Koch *et al*., 2017). Despite lacking statistical significance, higher DMI by treatment group cows in the prepartum period has substantial importance, as it was reported that each 0.1-percentage point reduction in the average DMI%BW and each 1-Mcal decline in the average energy balance in the last 3 d prepartum increased the odds of having clinical mastitis by 10 and 8%, respectively (Pérez-Báez, *et al.*, 2019). Moreover, it has been reported that decreased dry matter intakes, particularly in a week before calving, increased the risk of developing metritis in the postpartum period.

The lower postpartum concentration of NEFA in treatment group cows, especially in the first two weeks after calving, might be due to their higher prepartum dry matter intake, which reduced the mobilization of body fat by them. In addition, the positive effect of thyme on rumen fermentation and the ratio of acetate to propionate was reported before (Vakili *et al*., 2013; Castro Filho *et al*., 2021). Previous studies reported the association of high serum level peripartum NEFA and AST with increased risk of postpartum diseases in fresh cows. This elevated

risk of postpartum complications may be linked to the destructive effects of NEFA on neutrophils' function and viability (Hammon *et al*., 2006). Although impairment of neutrophils function under the presence of a high level of NEFA is proven, there is no consensus about the exact mechanism of NEFA-induced functional incompetency of polymerphonuclears. In contrast to previous reports about reduced ROS production and NETs formation, recent experiments revealed that overreacting of inflammatory pathways, overproduction of proinflammatory cytokines (Korbechi *et al*., 2019), and excessive production ROS in neutrophils may be the result of impaired function of neutrophils (Scalia *et* al., 2006; Zhang *et al*., 2018; Leblanc *et al*., 2020).

Regardless of the group, NEFA was higher in the first and second week postpartum compared to other time sessions. This is in accordance with a previous report about the presence of negative energy balance in the first three weeks after calving and lagging increase of DMI compared to energy demand. Similarly, irrespective of group, serum concentration of AST was higher in the first week postpartum than before calving and the third week postpartum, which may indicate hepatic damage due to metabolic load at the time of parturition. The effect of Echinacea extract itself on hepatic health is controversial. While there are some reports about the positive effect of Echinacea in the prevention and treatment of hepatic diseases (Manayi *et al*., 2015; Xu *et al.*, 2021), others reported hepatotoxicity caused by them (Abdel-Salam *et al*., 2012; Gabrains *et al*., 2015; Lawrenson *et al*., 2015). According to provided results, there was no significant increase in serum levels of ALT and AST during this study following Immunofin® supplementation. Although dosage and time length of treatment might play a role in how Echinacea extract affects the liver, lacking hepatic damage may occur because of hepatoprotective effects of rosemary and thyme (Domitrović *et al*., 2013; Rašković *et al*., 2015).

Provided results by our study demonstrate a lower count of peripheral blood leukocytes and lymphocytes in cows of the treatment group. Blood lymphocytes are the dominant subpopulation of an adult cow's normal leukogram (Roland *et al*., 2014). Since counts of other subpopulations of leukocytes didn't differ between the two groups of the study, lower leukocytes count in Immunofin® treated cows was due to a lower number of peripheral lymphocytes. Most of the previously conducted studies in other species reported an increased count of leukocytes following treatment with Echinacea extract (Mishima *et al*., 2004; Abouelella *et al*., 2007; Xu *et al*., 2021). Contrary to these results, there are several reports indicating the occurrence of leukopenia following long term treatment with Echinacea extract and the immunosuppressive effect of its lipophilic alkylamides through decreasing the count of lymphocytes (Kemp *et al*., 2002; Mattias *et al*., 2008; Balčiūnaitė-Murzienė *et al*., 2021). We did not observe any signs of leukopenia in treated cows, as their count of leukocytes was within the normal range. Considering this, a lower count of leukocytes among treated cows should be interpreted as the presence of leukocytosis among cows in the control group. This pathological leukocytosis might have occurred following the occurrence of clinical infectious diseases, including metritis among these cows. Regardless of group, peripheral blood leukocytes and lymphocytes were lesser in the first week postpartum than in the second session of sampling (two weeks after the beginning of the study) and third week postpartum. This changing pattern of WBCs and lymphocyte number might occur due to cortisol-induced stress leukogram at the time of parturition. It has been reported that NLR in the peripartum period is elevated among cows with a high level of SCC. The altered cytokines milieu and proinflammatory cytokines profile is considered a possible cause (Guan *et al*., 2020). Based on our findings, Immunofin® had no significant effect on NLR, but in contrast to the control group, the changing pattern of NLR in the treatment group was decreasing (Figure 3), which may decrease the risk of postpartum infectious disease diseases.

Reduction of MCH and MCV and reaching the lowest level in 30 days after calving in dairy cows was reported before (Painao, *et al*., 2020). The underlying pathophysiology might be due to an increased postpartum inflammatory state and intense body fat mobilization that suppress hematopoiesis due to iron deficiency (Ganz *et al.*, 2009; Contreras *et al*., 2011; Bradford *et al*., 2015). It has been reported that iron deficiency anemia impairs cell-

mediated immunity, and hence normal hematological indices are an important prerequisite of the competent immune response (Das *et al*., 2014). Mean corpuscular hemoglobin and mean corpuscular volume were significantly higher in the treatment group; this may indicate a controlled postpartum inflammatory state and reduced body fat mobilization among cows in the treatment group; a hypothesis which is supported by the evidence like lower WBCs in peripheral blood and lower serum NEFA concentration in these cows. These differences also could be explained by the hematopoietic effects of Echinacea. Similar effects were reported in other species treated by oral supplementation of Echinacea extract (O'Neill *et al*., 2002). Higher erythrogram in cows with better postpartum reproductive performance and lower erythrogram values in cows with retained fetal membrane was reported (Nazifi *et al*., 2008; Saut *et al*., 2008). Considering this, a lower incidence of RFM in the treatment group might be associated with Immunofin induced changes in the erythrogram.

Conclusion

Oral supplementation of Immunofin® polyherbal extract for three weeks in a close-up period TMR had no negative clinical impact on the health of postpartum Holstein dairy cows and elicited some positive effects, including significantly decreased NEFA and reduced incidence of postpartum uterine diseases.

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Conflict of Interest

The authors declared no conflict of interest.

References

Abdel-Salam, O. M., Sleem, A. A., El-Mosallamy, A. E., & Shaffie, N. (2012). Effect of Echinacea alone or in combination with silymarin in the carbon-tetrachloride model of hepatotoxicity. *Comparative Clinical Pathology*, *21*(6), 1483-1492. [DOI:10.1007/s00580-011-1317-1]

Alves, R. C., Duarte, D. L., David, T., & Silvia, A. (2016). Potential target for exploring medicinal plants use of bovine mastitis. *Int. J. Meth. Psychiatr. Res.*, *5*, 221-227.

Andrade, J. M., Faustino, C., Garcia, C., Ladeiras, D., Reis, C. P., & Rijo, P. (2018). Rosmarinus officinalis L.: an update review of its phytochemistry and biological activity. *Future Science OA*, *4*(4), FSO283. [DOI:10.4155/fsoa-2017-0124] [PMID] [PMCID]

Bicalho, M. L. S., Marques, E. C., Gilbert, R. O., & Bicalho, R. C. (2017). The association of plasma glucose, BHBA, and NEFA with postpartum uterine diseases, fertility, and milk production of Holstein dairy cows. *Theriogenology*, *88*, 270-282. [DOI:10.1016/j.theriogenology.2016.09.036] [PMID]

Bradford, B.J., Yuan, K., Farney, J.K., Mamedova, L.K., & Carpenter, A.J. (2015). Invited review: Inflammation during the transition to lactation: New adventures with an old flame. *Journal of Dairy Science, 98*(10), 6631-6650. [DOI:10.3168/jds.2015-9683] [PMID]

Bruni, R., Brighenti, V., Caesar, L. K., Bertelli, D., Cech, N. B., & Pellati, F. (2018). Analytical methods for the study of bioactive compounds from medicinally used Echinacea species. *Journal of Pharmaceutical and Biomedical Analysis*, *160*, 443-477. [DOI:10.1016/j.jpba.2018.07.044] [PMID]

Castro Filho, E. S., Júnior, L. C. R., Ezequiel, J. M. B., Salles, M. S. V., Almeida, M. T. C., Perez, H. L., ... & van Cleef, E. H. C. B. (2021). Effect of thyme essential oil supplementation on feed intake, apparent digestibility, rumen fermentation, blood parameters and in vitro

methane yield of Nellore cattle. *Livestock Science*, *244*, 104349. [DOI:10.1016/j.livsci.2020.104349]

Chebel, R. C. (2021). Predicting the risk of retained fetal membranes and metritis in dairy cows according to prepartum hemogram and immune and metabolic status. *Preventive Veterinary Medicine*, *187*, 105204. [DOI:10.1016/j.prevetmed.2020.105204] [PMID]

Contreras, G. A., & Sordillo, L. M. (2011). Comparative Immunology, Microbiology and Infectious Diseases Lipid mobilization and inflammatory responses during the transition period of dairy cows. *Comparative Immunology, Microbiology & Infectious Diseases.[Internet]. Elsevier Ltd*, *34*, 281-9. [DOI:10.1016/j.cimid.2011.01.004] [PMID]

Cui, D., Wang, S., Wang, L., Wang, H., Li, X., & Liu, Y. (2015). Prophylactic strategy with herbal remedy to reduce puerperal metritis risk in dairy cows: A randomized clinical trial. *Livestock Science*, *181*, 231-235. [DOI:10.1016/j.livsci.2015.09.005]

Cui, L., Wang, H., Ding, Y., Li, J., & Li, J. (2019). Changes in the blood routine, biochemical indexes and the pro-inflammatory cytokine expressions of peripheral leukocytes in postpartum dairy cows with metritis. *BMC Veterinary Research*, *15*(1), 1-10. [DOI:10.1186/s12917-019-1912-y] [PMID] [PMCID]

Das, I., Saha, K., Mukhopadhyay, D., Roy, S., Raychaudhuri, G., Chatterjee, M., & Mitra, P. K. (2014). Impact of iron deficiency anemia on cell-mediated and humoral immunity in children: A case control study. *Journal of Natural Science, Biology, and Medicine*, *5*(1), 158. [DOI:10.4103/0976-9668.127317] [PMID] [PMCID]

Dervishi, E., Zhang, G., Hailemariam, D., Dunn, S. M., & Ametaj, B. N. (2016). Occurrence of retained placenta is preceded by an inflammatory state and alterations of energy metabolism in transition dairy cows. *Journal of Animal Science and Biotechnology*, *7*(1), 1-13. [DOI:10.1186/s40104-016-0085-9] [PMID] [PMCID]

Domitrović, R., Škoda, M., Marchesi, V. V., Cvijanović, O., Pugel, E. P., & Štefan, M. B. (2013). Rosmarinic acid ameliorates acute liver damage and fibrogenesis in carbon tetrachloride-intoxicated mice. *Food and Chemical Toxicology*, *51*, 370-378. [DOI:10.1016/j.fct.2012.10.021] [PMID]

Gabranis, I., Koufakis, T., Papakrivos, I., & Batala, S. (2015). Echinacea-associated acute cholestatic hepatitis. *Journal of Postgraduate Medicine*, *61*(3), 211. [DOI:10.4103/0022-3859.159430] [PMID] [PMCID]

Ganz, T., & Nemeth, E. (2009, October). Iron sequestration and anemia of inflammation. In *Seminars in Hematology* (Vol. 46, No. 4, pp. 387-393). WB Saunders. [DOI:10.1053/j.seminhematol.2009.06.001] [PMID] [PMCID]

Gedikoğlu, A., Sökmen, M., & Çivit, A. (2019). Evaluation of Thymus vulgaris and Thymbra spicata essential oils and plant extracts for chemical composition, antioxidant, and antimicrobial properties. *Food Science & Nutrition*, *7*(5), 1704-1714. [DOI:10.1002/fsn3.1007] [PMID] [PMCID]

González, F. D., Muiño, R., Pereira, V., Campos, R., & Benedito, J. L. (2011). Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows. *Journal of Veterinary Science*, *12*(3), 251-255. [DOI:10.4142/jvs.2011.12.3.251] [PMID] [PMCID]

Guan, R. W., Wang, D. M., Wang, B. B., Jiang, L. Y., & Liu, J. X. (2020). Prognostic potential of pre-partum blood biochemical and immune variables for postpartum mastitis risk in dairy cows. *BMC Veterinary Research*, *16*(1), 1-11. [DOI:10.1186/s12917-020-02314-6] [PMID] [PMCID]

Hashemzadeh-Cigari, F., Khorvash, M., Ghorbani, G. R., Kadivar, M., Riasi, A., & Zebeli, Q. (2014). Effects of supplementation with a phytobiotics-rich herbal mixture on performance, udder health, and metabolic status of Holstein cows with various levels of milk somatic cell counts. *Journal of Dairy Science*, *97*(12), 7487-7497. [DOI:10.3168/jds.2014-7989] [PMID]

Kirkham, T. C. (2005). Endocannabinoids in the regulation of appetite and body weight. *Behavioral Pharmacology*, *16*(5-6), 297-313. [DOI:10.1097/00008877-200509000-00004] [PMID]

Koch, M. (2017). Cannabinoid receptor signaling in central regulation of feeding behavior: a mini-review. *Frontiers in Neuroscience*, *11*, 293. [DOI:10.3389/fnins.2017.00293] [PMID] [PMCID]

Korbecki, J., & Bajdak-Rusinek, K. (2019). The effect of palmitic acid on inflammatory response in macrophages: an overview of molecular mechanisms. *Inflammation Research*, *68*(11), 915-932. [PMID] [PMCID] [DOI:10.1007/s00011-019-01273-5]

Lawrenson, J. A., Walls, T., & Day, A. S. (2014). Echinacea-induced acute liver failure in a child. *J Paediatr Child Health*, *50*(10), 841. [DOI:10.1111/jpc.12726] [PMID]

LeBlanc, S. (2010). Monitoring metabolic health of dairy cattle in the transition period. *Journal of Reproduction and Development*, *56*(S), S29-S35. [DOI:10.1262/jrd.1056S29] [PMID]

LeBlanc, S. J. (2020). Relationships between metabolism and neutrophil function in dairy cows in the peripartum period. *Animal*, *14*, s44-s54. [DOI:10.1017/S1751731119003227] [PMID]

Lopreiato, V., Mezzetti, M., Cattaneo, L., Ferronato, G., Minuti, A., & Trevisi, E. (2020). Role of nutraceuticals during the transition period of dairy cows: A review. *Journal of Animal Science and Biotechnology*, *11*(1), 1-18. [DOI:10.1186/s40104-020-00501-x] [PMID] [PMCID]

Ma, Y., Feng, Y., Song, L., Li, M., Dai, H., Bao, H., ... & Liang, Y. (2021). Green tea polyphenols supplementation alters immunometabolism and oxidative stress in dairy cows with hyperketonemia. *Animal Nutrition*, *7*(1), 206-215. [DOI:10.1016/j.aninu.2020.06.005] [PMID] [PMCID]

Manayi, A., & Tehranifard, A. (2015). Modulation of liver enzymes by an Iranian preparation of Echinacea purpurea. *Research Journal of Pharmacognosy*, *2*(4), 1-3.

Moretti, P., Probo, M., Cantoni, A., Paltrinieri, S., & Giordano, A. (2016). Fluctuation of neutrophil counts around parturition in Holstein dairy cows with and without retained placenta. Research in veterinary science, 107, 207-212. [DOI:10.1016/j.rvsc.2016.06.015] [PMID]

Nazifi, S., Ahmadi, M. R., & Gheisari, H. R. (2008). Hematological changes of dairy cows in postpartum period and early pregnancy. *Comparative Clinical Pathology*, *17*(3), 157-163. [DOI:10.1007/s00580-008-0730-6]

O'neill, W., McKee, S., & Clarke, A. F. (2002). Immunological and haematinic consequences of feeding a standardised Echinacea (Echinacea angustifolia) extract to healthy horses. *Equine Veterinary Journal*, *34*(3), 222-227. [DOI:10.2746/042516402776186001] [PMID]

Pérez-Báez, J., Risco, C. A., Chebel, R. C., Gomes, G. C., Greco, L. F., Tao, S., ... & Galvão, K. N. (2019). Association of dry matter intake and energy balance prepartum and postpartum with health disorders postpartum: Part II. Ketosis and clinical mastitis. *Journal of Dairy Science*, *102*(10), 9151-9164. [DOI:10.4102/jsava.v91i0.1975] [PMID] [PMCID]

Pérez-Báez, J., Risco, C.A., Chebel, R.C., Gomes, G.C., Greco, L.F., Tao, S., … & Galvão, K.N. (2019). Association of dry matter intake and energy balance prepartum and postpartum with health disorders postpartum: Part II. Ketosis and clinical mastitis. *Journal of Dairy Science, 102*(10), 9151-9164. [DOI:10.3168/jds.2018-15879] [PMID]

Qu, Y., Fadden, A.N., Traber, M.G., & Bobe, G. (2014). Potential risk indicators of retained placenta and other diseases in multiparous cows. *Journal of Dairy Science, 97*(7), 4151-4165. [DOI:10.3168/jds.2013-7154] [PMID]

Raduner, S., Majewska, A., Chen, J. Z., Xie, X. Q., Hamon, J., Faller, B., ... & Gertsch, J. (2006). Alkylamides from Echinacea are a new class of cannabinomimetics: cannabinoid type 2 receptor-dependent and-independent immunomodulatory effects. *Journal of Biological Chemistry*, *281*(20), 14192-14206. [DOI:10.1074/jbc.M601074200] [PMID]

Rašković, A., Pavlović, N., Kvrgić, M., Sudji, J., Mitić, G., Čapo, I., & Mikov, M. (2015). Effects of pharmaceutical formulations containing thyme on carbon tetrachloride-induced liver injury in rats. *BMC Complementary and Alternative Medicine*, *15*(1), 1-11. [DOI:10.1186/s12906-015-0966-z] [PMID] [PMCID]

Saut, J. P. E., & Birgel Junior, E. H. (2008). Influência da retenção dos anexos fetais no hemograma de fêmeas bovinas da raça Holandesa. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *60*, 1315-1322. [DOI:10.1590/S0102-09352008000600004]

Scalia, D., Lacetera, N., Bernabucci, U., Demeyere, K., Duchateau, L., & Burvenich, C. (2006). In vitro effects of nonesterified fatty acids on bovine neutrophils oxidative burst and viability. *Journal of Dairy Science*, *89*(1), 147-154. [DOI:10.3168/jds.S0022-0302(06)72078-1]

Seckin, C., Alpun Kalayci, G., Turan, N., Yilmaz, A., Cizmecigil, U. Y., Aydin, O., ... & Yilmaz, H. (2018). Immunomodulatory effects of Echinacea and Pelargonium on the innate and adoptive immunity in calves. *Food and Agricultural Immunology*, *29*(1), 744-761. [DOI:10.1080/09540105.2018.1444738]

Seifi, H. A., LeBlanc, S. J., Leslie, K. E., & Duffield, T. F. (2011). Metabolic predictors of post-partum disease and culling risk in dairy cattle. *The Veterinary Journal*, *188*(2), 216-220. [DOI:10.1016/j.tvjl.2010.04.007] [PMID]

Shi, Z., Li, X. B., Peng, Z. C., Fu, S. P., Zhao, C. X., Du, X. L., ... & Li, X. W. (2018). Berberine protects against NEFA-induced impairment of mitochondrial respiratory chain function and insulin signaling in bovine hepatocytes. *International Journal of Molecular Sciences*, *19*(6), 1691. [DOI:10.3390/ijms19061691] [PMID] [PMCID]

Skibiel A.L. , Zachut M. , Amaral B. C. , Levin Y. , Dah G.E. . Liver proteomic analysis of postpartum Holstein cows exposed to heat stress or cooling conditions during the dry period. *Journal of Dairy Science, 101*(1) ,705-716. [DOI:10.3168/jds.2017-13258] [PMID]

Song, Y., Loor, J. J., Zhao, C., Huang, D., Du, X., Li, X., ... & Li, X. (2020). Potential hemo-biological identification markers to the left displaced abomasum in dairy cows. *BMC Veterinary Research*, *16*(1), 1-9. [PMCID] [DOI:10.1186/s12917-020-02676-x] [PMID]

Stevenson, J. S., Banuelos, S., & Mendonça, L. G. (2020). Transition dairy cow health is associated with first postpartum ovulation risk, metabolic status, milk production, rumination, and physical activity. *Journal of Dairy Science*, *103*(10), 9573-9586. [DOI:10.3168/jds.2020-18636] [PMID]

Vakili, A. R., Khorrami, B., Mesgaran, M. D., & Parand, E. (2013). The effects of thyme and cinnamon essential oils on performance, rumen fermentation and blood metabolites in Holstein calves consuming high concentrate diet. *Asian-Australasian Journal of Animal Sciences*, *26*(7), 935. [DOI:10.5713/ajas.2012.12636] [PMID] [PMCID]

Xu, W., Zhu, H., Hu, B., Cheng, Y., Guo, Y., Yao, W., & Qian, H. (2021). Echinacea in hepatopathy: A review of its phytochemistry, pharmacology, and safety. *Phytomedicine*, *87*, 153572. [DOI:10.1016/j.phymed.2021.153572] [PMID]

Zhang, Y., Li, X., Zhang, H., Zhao, Z., Peng, Z., Wang, Z., ... & Li, X. (2018). Non-esterified fatty acids over-activate the TLR2/4-NF-κb signaling pathway to increase inflammatory cytokine synthesis in neutrophils from ketotic cows. *Cellular Physiology and Biochemistry*, *48*(2), 827-837. [DOI:10.1159/000491913] [PMID].

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مجله طب دامی ایران، ۱۴۰۱، دوره ۱۶، شماره ۳، ۲۷۴-۲۸۷

ارزیابی پارامترهای بیوشیمیایی و هماتولوژیک در گاوهای هلشتاین تازه زا پس از افزودن عصاره گیاهی ایمونوفین به جیره

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زمینه مطالعه: بروز بیماری‌ها در گاوهای شیری در دوره پس از زایش زیاد است و این امر منجر به کاهش سودآوری گله‌های گاو شیری و افزایش نیاز به مصرف آنتی‌بیوتیک‌ها می‌گردد. با توجه به این امر، ایده بهبود سلامت و کاهش مصرف آنتی‌بیوتیک‌ها در طب گاو شیری با بهره بردن از ترکیبات زیست فعال موجود در عصاره‌های گیاهی رو به گسترش است.

هدف: هدف اصلی این مطالعه بررسی تاثیر اضافه نمودن عصاره گیاهی آبی ایمونوفین (شرکت پارس ایمن دارو) به خوراک آن‌ها در دوره کلوزآپ بر فیزیولوژی طبیعی، سلامت بالینی و پارامترهای بیوشیمیایی و هماتولوژیک گاوهای تازه‌زا است.

روش کار: در این مطالعه، طی دوره کلوزآپ ایمونوفین به خوراک گاوهای تیمار (۱۰ راس) اضافه شد درحالی‌که گاوهای گروه شاهد (۱۰ راس) جیره‌ای مشابه را با دارونما دریافت کردند. سلامت بالینی گاوها و شاخص‌های بیوشیمیایی و هماتولوژیک بین دو گروه مقایسه شد.

نتایج: نرخ بروز جفت ماندگی و متریت در گاوهای گروه تیمار کمتر از گروه شاهد بود ($P=۰/۰۱$) به علاوه غلظت اسیدهای چرب غیر استریفیه در گروه درمان پایین‌تر از گروه کنترل بود ($P<۰/۰۰۱$). گاوهای گروه تیمار لمفوسیت‌ها و لوکوسیت‌های کمتری در خون خود داشتند ($P<۰/۰۰۰۱$) درحالی‌که وزن متوسط هموگلوبین گویچه‌ای و حجم متوسط گلبول‌های قرمز در آن‌ها بیشتر بود ($P<۰/۰۰۰۱$).

نتیجه‌گیری نهایی: اضافه نمودن ایمونوفین به جیره دوره کلوزآپ گاوها نه تنها اثری منفی بر سلامت بالینی آن‌ها ندارد بلکه می‌تواند برخی از شاخص‌های بالینی، بیوشیمیایی و هماتولوژیک گاوهای تازه‌زا را بهبود بخشد.

واژه‌های کلیدی: سلامت بالینی، ایمونوفین، وضعیت متابولیک، عصاره گیاهی، دوره انتقال

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