ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

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The Efficacy of Zeolite Supplementation on Milk Yield, Hematological and Serum Biochemical Parameters in Holstein Cows

Holstein İneklerde Zeolit Desteğinin Süt Verimi, Hematolojik ve Serum Biyokimyasal Parametreleri Üzerine Etkisi

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ABSTRACT This research was conducted to analyze of the influence of zeolite administration on milk yield, hematological and biochemical parameters in Holstein cows. The animal material of the study constituted 30 head Holstein cows resided in a private cattle practice farm in Aydin municipality, Bozdogan whereas the food stuff were composed of 0%, 1.25% and 2.5% natural zeolite (clinoptilolite) involved feed mixture. This research, included a total of 3 groups as control (10 head), Zeolite I (10 head), Zeolite II (10 head). As in agreement with the purpose of the present study, the influence of zeolite on hematological and serum biochemical alterations, was analyzed by blood samples obtained at 4 different periods. Regarding daily milk yield, Zeolite groups I and II revealed 2,38 kg and 3,29 kg increases, respectively, in comparison to the control group with a statistically significant importance ($p<0.01$). Hematological parameters were within the reference ranges in groups in early lactation (RBC) ($p<0.01$) and mid-lactation (RBC and WBC) ($p<0.05$). During ration adaptation period and beginning of the lactation, a marked alteration ($p<0.01$) was detected among groups with regard to AST and creatinine parameters, whereas glucose at mid-lactation ($p<0.01$) ALT and urea at closing lactation ($p<0.05$) possessed significant alterations among study groups. In conclusion through evaluated hematological and serum biochemical parameters, dietary added zeolite did not cause side effects and long-term zeolite administration did not influence normal physiological homeostasis in animals enrolled.

Keywords: Zeolite; Holstein; hematological; biochemical; parameters

ÖZET Bu araştırma, Holstein ineklerinde zeolit uygulamasının süt verimi, hematolojik ve biyokimyasal parametreler üzerindeki etkisinin analiz edilmesi amacıyla yapılmıştır. Çalışmanın hayvan materyali Bozdogan Aydın belediyesinde özel bir sığırcılık işletmesinde yaşayan 30 baş Holstein ineği oluşturmakta ve rasyonlarında %0,%1,25 ve %2,5 doğal zeolit (klinoptilolit) içeren gıda maddeleri yem karışımını içermektedir. Bu araştırma kontrol (10 baş), zeolit I (10 baş), zeolit II (10 baş) olmak üzere toplam 3 grup içermektedir. Bu çalışmanın amacına uygun olarak zeolitin hematolojik ve serum biyokimyasal değişiklikler üzerindeki etkisi, 4 farklı dönemde elde edilen kan örnekleri ile analiz edildi. Günlük süt verimi bakımından, zeolit grupları I ve II, kontrol grubuna kıyasla sırasıyla 2,38 kg ve 3,29 kg artış gösterdi ve bu anlamlılık istatistiksel olarak önemliydi ($p<0,01$). Hematolojik parametreler erken laktasyon (RBC) ($p<0,01$) ve orta laktasyon (RBC ve WBC) ($p<0,05$) gruplarında referans aralıkları içerisindeydi. Rasyon adaptasyon döneminde ve laktasyonun başlangıcında, AST ve kreatinin parametreleri ile gruplar arasında belirgin bir değişiklik ($p<0,01$) bulunurken, orta laktasyonda glukoz ($p<0,01$) ALT ve kapanış laktasyonunda üre ($p<0,05$) çalışma grupları arasında önemli değişikliklere sahipti. Sonuç olarak rasyona katılan zeolitin hematolojik ve serum biyokimyasal parametrelerini etkilemediği ve uzun süre kullanımının da çalışmada kullanılan hayvanların normal fizyolojik hemostazlarını değiştirmediği belirlendi.

Anahtar Kelimeler: Zeolit; Holstein; hematolojik; biyokimyasal; parametreler

Zeolites, hydrated aluminosilicates, recognized as "molecular sieves" with a formula of (Na4K4) (Al8Si40O96)×24 H2O, are able to present crystalized arrangement.[1] Due to their cationic binding properties, they may be beneficial for preventing animals from the filigree build up of toxicological material.[2]

Available evidence suggested the favorable efficacy of the clinoptilolite addendum for the diet regarding the prosperity of animals and in prevention against ammonium poisoning.[3,4]

Regarding agricultural engineering and veterinary sciences for dairy cattle, evaluation of hematological and biochemical biomarkers for metabolic testing, has pivotal role in health status, improvement in milk yield, and reproduction.[5-7]

The present study was performed in an attempt to evalaute the influence of clinoptilolite from Aydin province, Turkey in Holstein cows for determining the effects on milk yield, body condition scoring, and on hematological and selected serum biochemical components. Another purpose was to analyze whether the supplementation of clinoptilolite to cow diets at different levels of 0%, 1.25% or 2.5% would positively affect milk yield, body condition scoring, hematological and serum biochemical homeostasis of animals during several lactational duration.

MATERIAL AND METHODS

ANIMALS AND ZEOLITE MATERIAL

The present research was conducted with thirty healthy head Holstein-Friesian cows (among 1st and 4rd lactation) in a commercial dairy farm located at the Eagean Region of Turkey. Natural zeolite compound, clinoptilolite, was obtained from a commercial company (Gordes Zeolite Mining, İzmir Turkey). Regarding clinoptilolite, NH4 ion exchange capacity and the present formulation were similar to a previous study of the authors.[8] This study was performed in accordance with the principles of Helsinki Declaration and all animal owners in the study were informed. This study was approved by Aydın Adnan Menderes University Animal Ethics Comitee (64583101/2013/076).

EXPERIMENTAL DIETS

In the present research, cows were enrolled into 3 different groups (Table 1): Group I (control group) included 10 cows which received a basal diet (Table 2), Group II (Zeolite cows) included the animals (n=10) which were adminestered a basal diet+1.25% zeolite, and Group III (Zeolite II cows) (n=10) included the cows which received a basal diet +2.5% zeolite. The visual form of zeolite is given in Figure 1.

TABLE 1: Demographic data of groups.

Group I (n=10)	Control group comprising 10 cows, which received basal diet
Group II (n=10)	Zeolite cows which received a basal diet +1.25% zeolite
Group III (n=10)	Zeolite II cows (n=10) which received a basal diet +2.5% zeolite

TABLE 2: Composition of basal diet.

Ingredient	DM%
Sugar beet pulp	25
Hay	19
Maize grains	5
Barley grains	12
Cottonseed oil meal	5
Sunflower seed meal	5
Wheat bran	25
Molasses	3
Limestone	0.4
Salt	0.4
Vitamin-mineral premix	0.2
Chemical composition	
Dry matter intake/day (kg)	16.00
Crude protein (DM %)	16.06
NE [Net energy lactation (Mcal/kg DM)]	1.48
Ca (DM %)	0.38

Contained in kg og vitamin-mineral premix: Vitamin A 90.000 UI; Vitamin D 6000 UI; Vitamin E 60 mg; Vitamin PP 900 mg; Vitamin B1 7.50 mg; Vitamin B2 7.50 mg; Zinc 240 mg kg^{-1}; Iron 150 mg kg^{-1}; Selenium 1 mg kg^{-1}; Iodium 3 mg kg^{-1}.
DM: Dry matter.

EXPERIMENTAL DESIGN

The diet practice period was carried out for 15 days just prior to the trial. Animals were residing in field conditions. All cows were fed daily at 09:00 and 16:00 h in barn. The hay was available ad-libitum for whole trial period and concentrate feed was given to the mean values milk yields of groups. The Zeolite I and II groups received an equal diet, besides 1.25% and 2.5%, zeolite were added,

FIGURE 1: The visual form of zeolite used in the present study.

respectively. All cows were freely entered to water consumption. Milking procedure daily was done at 06:00 in the morning and at 17:00 in the afternoon. Milk yields were recorded daily throughout the experimental period (ration adaptation/practice period, early, mid and late lactation). Taking into account the relevant literature, lactation period was denoted as the first 2 (early lactation); 2-7 (mid-lactation); 7-10 (late lactation) months of lactation.[7]

BODY CONDITION SCORE

The body condition score (BCS) was calculated entirely upon observational estimation [via usage of a 1-5 scale] similar to what have been reported elsewhere.[8,9] Following morning milking procedure, BCS data were calculated for the whole trial period. Table 2 presents related data.

HEMATOLOGICAL AND SERUM BIOCHEMICAL ANALYSIS

Regarding evaluation of the efficacy of zeolite on hematological analysis comprising complete blood count with Abacus Junior hematology analyzer and the serum concentrations of urea, creatinine, triglyceride and activity of transaminases, namely ALT and AST, were established on a clinical chemistry auto-analyzer (Samsung) with commercial test kits. Blood was drawn from *Vena cephalica antebrachii* for a total of 4 times at ration adaptation, early, mid and late lactation.[7]

MILK YIELD

The 305-day milk yields were calculated by using suitable coefficients from the lactation milk yields of the cows in the present study, as previously described.[10] The animal materials were distributed by parity. The first 2 groups involved first, second and third lactation cows whereas the last group involved fourth and more lactating cows.

STATISTICAL ANALYSIS

Data analysis composed of ANOVA [via General Linear Model (GLM)] procedure of the statistical program SPSS version 17.0 for Windows.[11] The efficacy of zeolite supplementation was analyzed by Repeated Measures Analysis. The differences between subclass means were determined by Duncan's multiple range test.[12] The statistical models practiced for the study were:

Model 1 $Y_{ijklm}= m+b_i+ls_j+ lp_k+ z_l+ e_{ijklm}$

where, Y_{ijklm}: i. calving month, j. parity, k. lactation period, l. zeolite groups, m. cow's milk yield,

Model 2 $Y_{ijk}=m+lp_i+z_j+(ldz)_{ij}+e_{ijk}$

where, Y_{ijk}: i. lactation period, j. zeolite groups, k. cow's haematologic and biochemical parameters,

Model 3 $Y_{ijk}=m+lp_i+z_j+(ldz)_{ij}+e_{ijk}$

where, Y_{ijk}: i. lactation period, j. zeolite groups, k. cow's body condition score, m: population mean, b_i: i. calving month's effect (i: 1, 2, 3, 4), ls_j: j. parity's effect (1, 2, 3, 4), lp_k ve lp_i: lactation period's effect [1: practice period (first 15 days)], 2: beginning lactation (0-70 days), 3: middle lactation period (71-140. days), 4: late lactation period (141. day and later), z_j ve z_l: zeolite group's effect (1: Control group, 2: 1.25% zeolite, 3: 2.5% zeolite), $(ldz)_{ij}$: Lactation period X zeolite group interaction and eijkl: Residual error.

RESULTS

HEMATOLOGICAL RESULTS

Table 3 shows hematological parameters (WBC, RBC, HGB, MCV, and MCHC) based on 4 different period and group interactions. As shown in Table 2, WBC values varied between 10.99-14.28 (10^9/L), 11.20-14.48 6.46-8.71 (10^9/L), and 7.44-7.58 (10^9/L) in (10^9/L) in ration adaptation, early, mid and late lactation periods, respectively. There was a significant alteration ($p<0.05$) among groups in mid-lactation period. Period X group interaction

TABLE 3: The least square means and standard variations of hematological parameters among different periods and trial groups.

Stages	Parameters	Control N=10	Zeolite I (1.25%) N= 10	Zeolite II (2.5%) N= 10	p	Periods X Groups
Ration adaptation	WBC	14.28±2.494	10.99± 1.850	13.19±2.097	N.S.	N.S.
	RBC	5.95 ± 0.346	5.05±0.491	5.47±0.238	N.S..	N.S.
	Hb	10.75± 1.364	9.03±0.794	11.87±0.639	N.S.	*
	MCV	58.00± 3.765	56.30±3.425	64.60±1.194	N.S.	*
	MCHC	30.34± 3.404	32.80± 0.351	33.51±0.439	N.S.	**
Early lactation	WBC	14.48± 2.479	11.20± 5.762	13.41±2.045	N.S.	N.S.
	RBC	8.14^{a}± 0.401	5.76^{b}±0.691	6.10b±0.233	N.S.	N.S.
	Hb	11.60± 1.049	9.41±0.805	11.87±0.630	N.S.	N.S.
	MCV	60.44± 3.690	57.85± 3.281	65.20±1.051	N.S.	**
	MCHC	33.82± 0.637	33.44± 0.448	33.10±0.385	N.S.	*
Mid-lactation	WBC	8.71^{a}± 0.583	6.46^{b} ± 0.441	8.64^{a}±0.957	*	N.S.
	RBC	6.76^{a}± 0.383	7.19^{ab}± 0.444	8.17^{b}±0.359	*	N.S.
	Hb	8.30 ± 0.589	9.84 ± 0.410	8.73± 0.556	N.S.	N.S.
	MCV	36.70± 1.693	37.50± 1.565	38.10±1.441	N.S.	*
	MCHC	29.48± 2.317	32.11± 0.940	31.88±0.805	N.S.	*
Late lactation	WBC	7.44 ± 0.836	7.58 ± 1.112	7.48±2.248	N.S.	*
	RBC	7.88 ± 1.653	6.85 ± 1.248	6.45±1.270	N.S.	N.S.
	Hb	9.82± 0.515	9.98 ± 0.452	8.31±0.612	N.S.	**
	MCV	37.90± 1.587	38.80± 1.562	37.00±1.693	N.S.	N.S.
	MCHC	31.83± 0.834	31.68±0.515	29.66±2.352	N.S.	*

^{a,b,c}: Means with different superscripts in each line are different; *: $p<0.05$; **: $p<0.01$; N.S.: Non-significant; WBC: Leukocyte count (10^9/L); RBC: Erythrocyte count ($x10^6/mm^3$); Hb: Haemoglobin (g/dl); MCV: mean corpuscular volume (fl); MCHC: mean corpuscular haemoglobin concentration (g/dl).

revealed that there was a significance ($p<0.05$) regarding intergroup interaction. This significance was derived from interaction between Zeolite I group and late lactation (Figure 2).

Red blood cell values showed similar results in ration adaptation period varying between 5.05-5.95 ($x10^6/mm^3$). Values ranged between 5.76-8.14 ($x10^6/mm^3$) in early lactation, 6.67-8.17 ($x10^6/mm^3$) in mid-lactation and 6.45-7.88 ($x10^6/mm^3$) in late lactation. Regarding RBC values, there was a group significance in early lactation ($p<0.01$) and mid-lactation ($p<0.05$).

Hemoglobin (Hb) values, were detected as 9.03-11.87 g/dl, 9.41–11.87 g/dl, 8.30-9.84 g/dl and 8.31-9.98 g/dl, in ration adaptation, early, mid and late lactation periods, respectively.

Regarding Hb values there was a group x period interaction significance in ration adaptation ($p<0.01$) and late lactation ($p<0.05$). This significance was related to the interaction between ration adaptation and control group, and late lactation period and Zeolite I group (Figure 3).

Mean corpuscular volume (MCV) values presented variances between 56.30-64.60 fl and 57.85-65.20 fl in ration adaptation and early lactation periods, respectively, whereas the latter valued declined into 36.70-38.10 fl and 37.00-38.80 fl in mid-lactation and late lactation, respectively. Group X period interactions revealed statistical significance among trial groups, other than late lactation period. There was significance among ration adaptation and control, Zeolite I groups ($p<0.05$). Besides, early lactation ($p<0.01$) and mid-lactation ($p<0.05$) revealed significant alterations in contrast to the control group (Figure 4).

Mean mean corpuscular hemoglobin concentration (MCHC) values showed similar values among 4 periods of the study. Group X

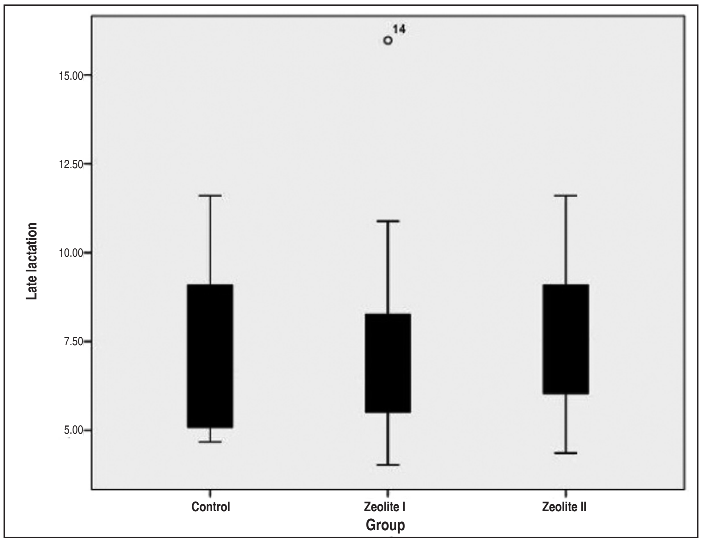

FIGURE 2: Alterations in WBC values during in late lactation period.

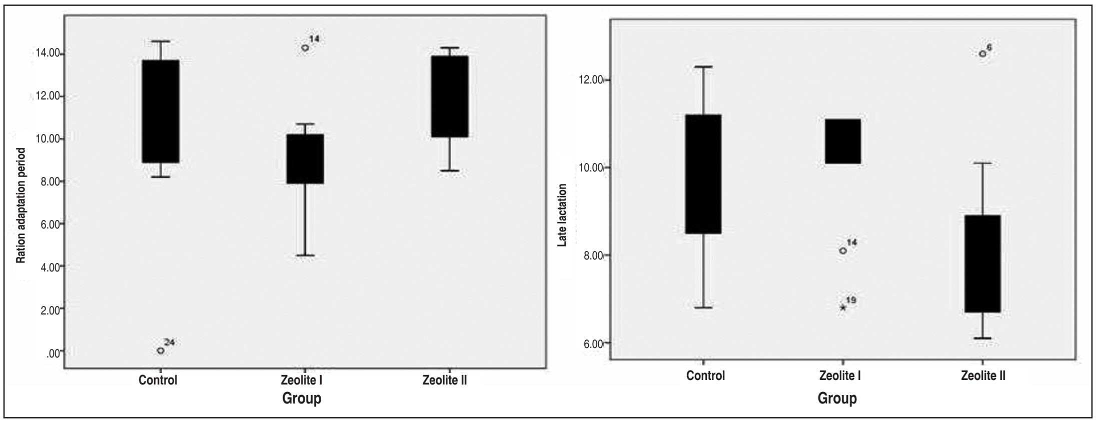

FIGURE 3: Alterations in Hb values during ration adaptation period and late lactation.

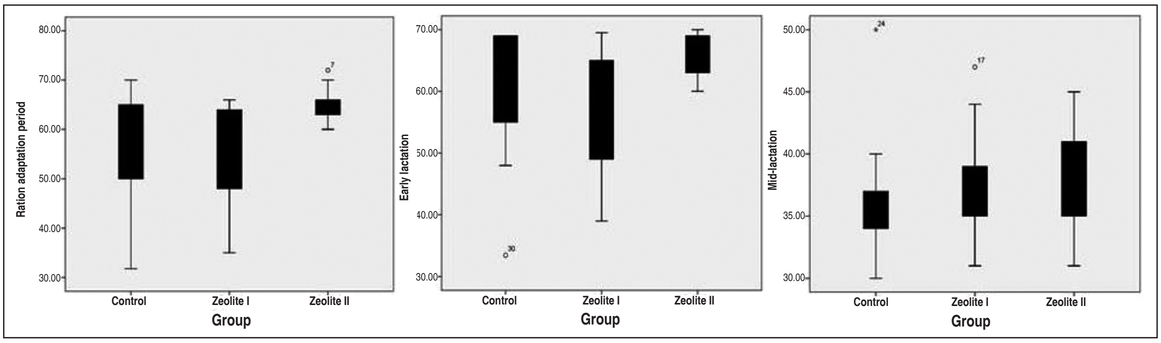

FIGURE 4: Alterations in MCV values during ration adaptation period, early and mid-lactation.

period interactions revealed significance among ration adaptation and control groups ($p<0.01$); early lactation and Zeolite I groups; mid-lactation and Zeolite I and Zeolite II groups, late lactation and Zeolite II groups ($p<0.05$) (Figure 5).

SERUM BIOCHEMICAL RESULTS

Serum biochemical parameters involved and analyzed in the present study are given in Table 4, among 4 different periods at each 3 sampling times of groups (Table 4).

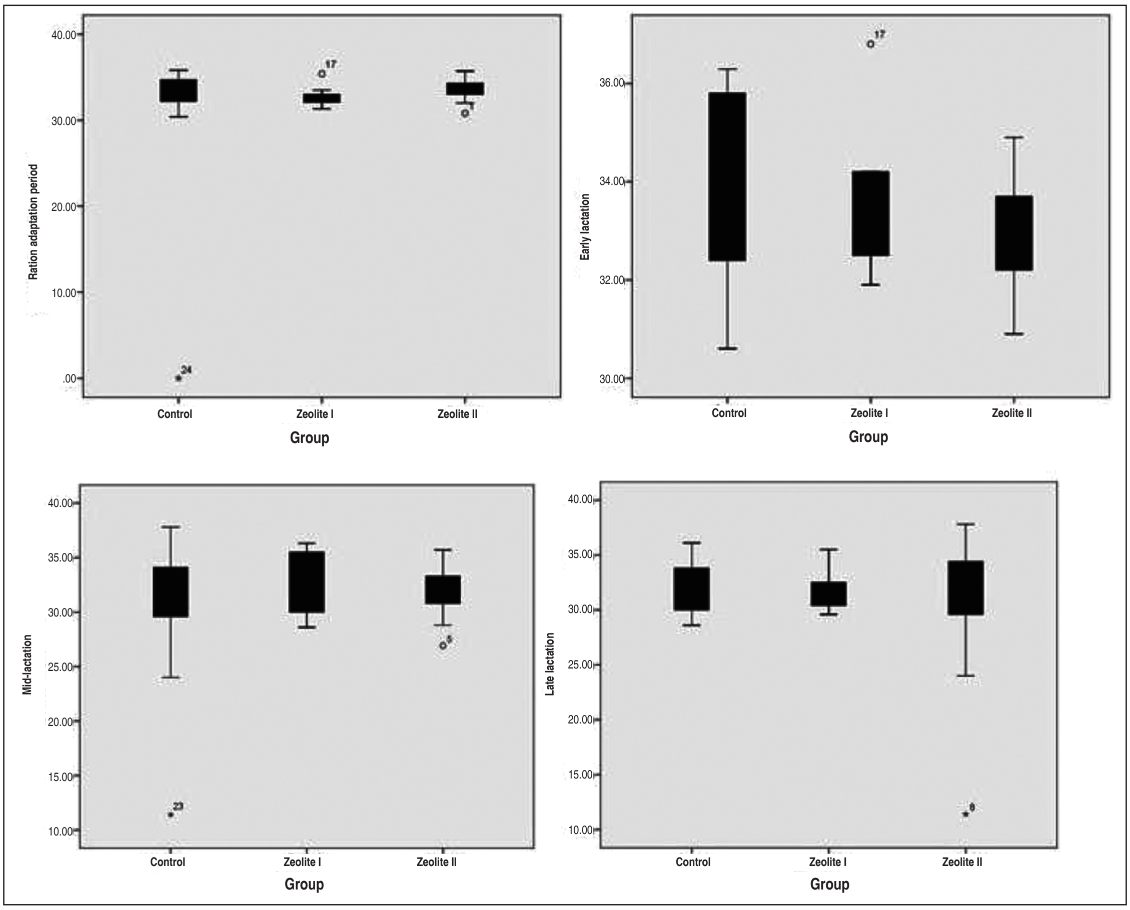

FIGURE 5: Alterations in MCHC values during ration adaptation and other lactation periods.

One of the indicators of liver activity i.e. ALT values presented variances between 24.40-28.60 U/L in ration adaptation, 27.70-36.80 U/L in early lactation, 18.80-21.90 U/L in mid-lactation and 18.60-23.30 U/L in late lactation. The differences between ALT values found to be significant in late lactation ($p<0.05$).

The values of mean AST were 65.40-92.60 U/L, 69.90-109.70 U/L, 39.40-40.30 U/L and 37.70-40.40 U/L through ration adaptation, early, mid and late lactation, respectively. The mean AST values of ration adaptation and early lactation stages were elevated in contrast to the other stages, Period X group interaction was evident ($p<0.05$), only in ration adaptation stage in Zeolite I group (Figure 6).

When glucose values were evaluated, there was fluctuation, as expected, presenting values between 63.30-66.60 mg/dl in ration adaptation, 57.30-65.50 mg/dl in early lactation, 44.10-55.50 mg/dl in mid-lactation and finally 48.80-50.90 mg/dl in late lactation. In mid-lactation, there was a significant difference among groups ($p<0.05$). Regarding period X group interaction, there was a significant alteration in mid-lactation and late lactation periods ($p<0.05$). The differences observed was among Zeolite I group in mid-lactation and control group in late lactation (Figure 7).

Urea levels were deemed 21.60-23.20 mg/dl in ration adaptation, 20.60–22.90 mg/dl in early lactation, 19.90-23.20 mg/dl in mid-lactation and 19.40-24.20 mg/dl in late lactation. At late lactation mean urea values presented significant differences, among groups ($p<0.05$). Regarding period X group interaction, there was a significance in early lactation ($p<0.05$) (Figure 8).

Creatinine values showed variances among groups detected between 0.33 -0.84 mg/dl in ration

TABLE 4: The least square means and standard variations of serum biochemical parameters among different periods and trial groups.

Periods	Parameters	Control N=10	Zeolite I (1.25%) N= 10	Zeolite II (2.5%) N= 10	p	Periods X Groups
Ration adaptation	ALT	24.40± 1.469	28.60 ± 2.765	24.50 ± 1.013	N.S.	N.S.
	AST	92.60^{a}± 1.64	84.30^{b} ± 4.716	65.40^{c} ± 2.012	**	*
	Glucose	66.60± 4.177	63.30 ± 19.177	64.88 ± 7.670	N.S.	N.S.
	Urea	21.60± 1.733	23.20 ± 1.518	23.20 ± 1.711	N.S.	N.S.
	Creatinine	0.44 ± 0.082	0.33 ± 0.064	0.84 ± 0.043	**	N.S.
	Triglycerid	8.73 ± 0.568	7.25 ± 0.509	8.30 ± 0.529	N.S.	N.S.
Early lactation	ALT	27.70 ± 2.12	36.80 ± 3.190	24.80 ± 3.000	N.S.	N.S.
	AST	104.40^{a}±4.602	109.70^{a}± 6.173	69.90^{b} ± 2.267	**	N.S.
	Glucose	65.50± 4.253	57.60 ± 6.718	57.30 ± 0.597	N.S.	N.S.
	Urea	20.60±1.309	22.90 ± 1.615	22.70 ± 1.598	N.S.	**
	Creatinine	0.42^{a} ± 0.070	0.43^{a} ± 0.085	0.79^{b} ± 0.044	**	N.S.
	Triglycerid	8.74 ± 0.533	7.29 ± 0.475	8.20 ± 0.492	N.S.	*
Mid-lactation	ALT	19.90 ± 1.656	21.90 ± 1.303	18.80 ± 1.171	N.S.	N.S.
	AST	40.30 ± 1.591	39.70 ± 1.453	39.40 ± 1.266	N.S.	N.S.
	Glucose	46.40^{a} ± 2.809	44.10^{a} ± 3.117	55.50^{b} ± 2.291	*	*
	Urea	20.50 ± 1.470	23.20 ± 1.083	19.90 ± 1.159	N.S.	N.S.
	Creatinine	0.82 ± 0.025	0.85 ± 0.028	0.88 ± 0.030	N.S.	N.S.
	Triglycerid	6.74 ± 0.565	6.60 ± 0.635	7.45 ± 0.623	N.S.	N.S.
Late lactation	ALT	18.90a ± 1.187	23.30^{b} ± 1.333	18.60^{a} ± 1.771	*	N.S.
	AST	37.80 ± 1.884	40.40 ± 1.343	37.70 ± 1.776	N.S.	N.S.
	Glucose	50.50 ± 3.754	50.90 ± 2.876	48.80 ± 3.319	N.S.	*
	Urea	22.60^{ab} ± 1.384	24.20^{a} ± 1.103	19.40^{b}± 0.921	*	N.S.
	Creatinine	0.82 ± 0.025	0.87 ± 0.044	0.89 ±0.036	N.S.	N.S.
	Triglycerid	8.45 ± 0.382	7.25 ± 0.663	6.86 ± 1.136	N.S.	**

a,b,c: Means with different superscripts in each line are different; *: p<0.05; **: p<0.01; N.S.: Non-significant; ALT: Alanine amino transferase (U/l); AST: Aspartate amino transferase (U/l).

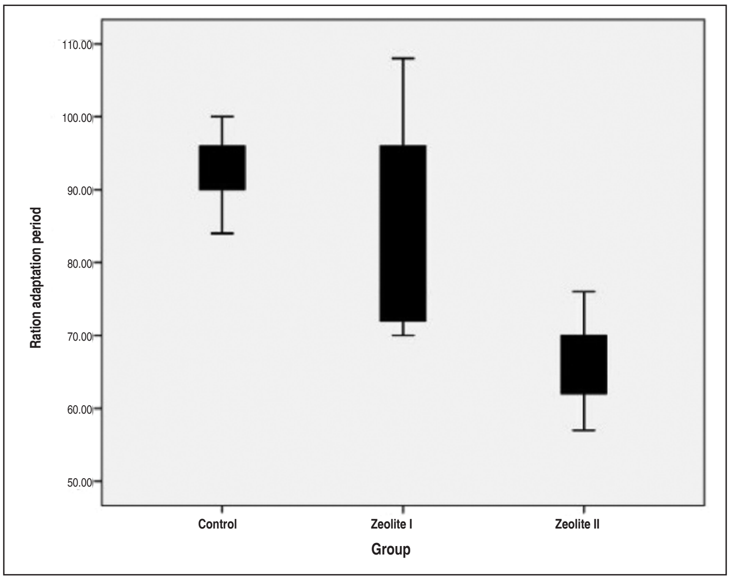

FIGURE 6: Alterations in AST values in ration adaptation.

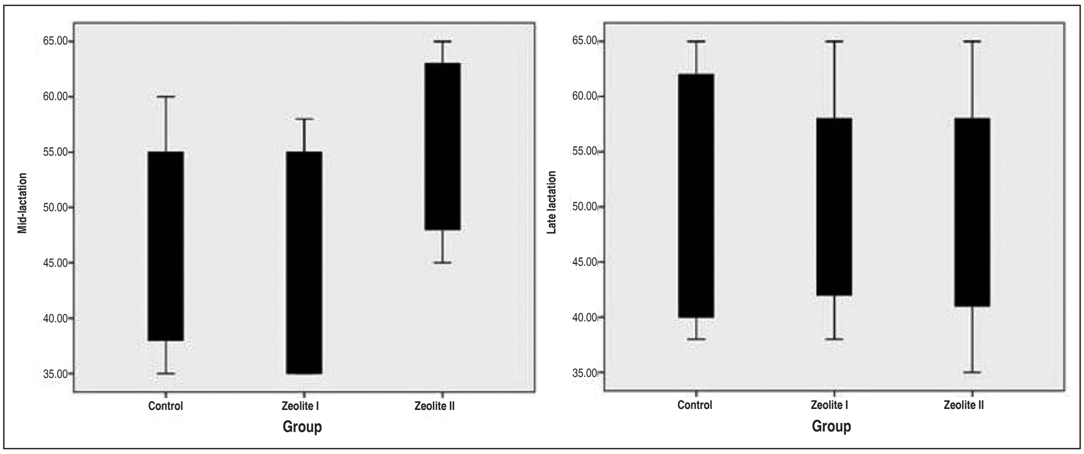

FIGURE 7: Alterations in glucose values in mid and late lactation.

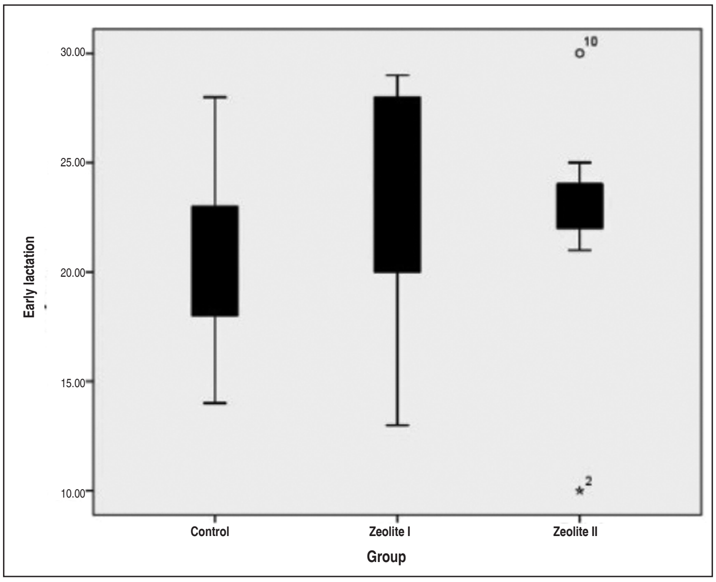

FIGURE 8: Alterations in urea values in early lactation.

adaptation period, 0.42-0.79 mg/dl in early lactation, 0.82-0.88 mg/dl in mid-lactation and 0.82-0.89 mg/dl in late lactation.

Group impact on creatinine values presented statistical significance in ration adaptation and early lactation ($p<0.01$). Besides, period X group interaction revealed non-significant alterations ($p>0.05$).

One of the important indicators of the lipid profile, i.e. triglycerides values showed ranges such as 7.25-8.73 mg/dl, 7.29-8.74 mg/dl, 6.60-7.45 mg/dl and 6.86-8.45 mg/dl in ration adaptation, early/mid and late lactation periods in this study. Obtained values presented non-significant ($p>0.05$) group comparison, whereas period X group interaction presented statistical significance in early lactation ($p<0.05$) and in late lactation ($p<0.01$). This significance was due to early lactation and late lactation periods between control and Zeolite II group (Figure 9).

MILK YIELD

The daily milk yields were found to show 2.38 kg and 3.29 kg increases, in both zeolite groups, respectively. The alterations among groups were found statistically significant ($p<0.01$).

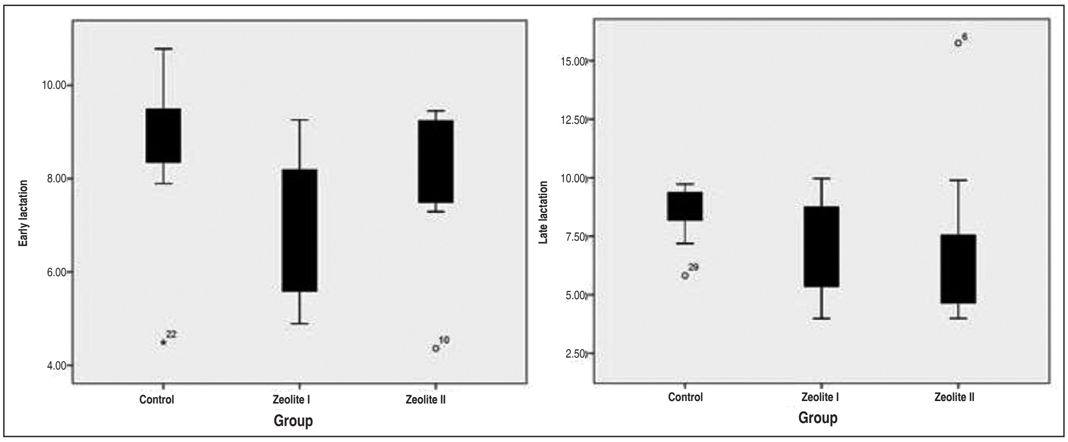

FIGURE 9: Alterations in triglyceride values in early and late lactation.

BODY CONDITION SCORE

It was detected that BCS values presented a tendency to increase at the end of lactation. The differences between groups with regard to BCS were found statistically non-significant (p>0.05), while significant (p<0.01) alterations were found between stages for each group (Table 5).

DISCUSSION

Hematological analysis for food animals involving short term or long-duration zeolite additive was the subject of some prior studies. Some researchers claimed that 3% addition to the diet caused no significant alteration for PCV (for 8 weeks) in contrast to control calves.[13] In a previous study, it was hypothesized that long term clinoptilolite supplementation (1.25% or 2.5%) to dairy cows resulted in no significant alterations among mean RBC or PCV values.[14]

A prior study investigating the effects of clinoptilolite in terms of hematology parameters to those of calves administered colostrum with additive (clinoptilolite as 5 and/or 10g/L milk) revealed that hematological biomarkers (RBC, Hb, and PCV) showed no deviations from physiological values. The researchers also proposed that zeolite did not influence the level of iron resorption rate or other relevant oligo elements deemed necessary for erythropoiesis.[15] In contrast, there was a group X period interaction significance regarding Hb values in ration adaptation (p<0.01) and late lactation (p<0.05) groups. This significance was related to the interaction between ration adaptation and control group, and late lactation period and Zeolite I group in the present study. In another trial investigating the effects of 80% clinoptilolite 20% sepiolite on milk yield and hematological parameters (for 12 weeks) suggested that there was no statistical significance (p>0.05) on Hb or MCV values.[16] In the present study, MCV values presented period X group interactions and there was significance among ration adaptation and control, Zeolite I groups (p<0.05). Besides, early lactation (p<0.01) and mid-lactation (p<0.05) revealed significant alterations. On the other hand, this study denoted that milk yield was higher [27.6 kg/day] in zeolite supplemented group whereas control group revealed 25.1 kg/day.

Previous literature suggested that supplemented short term (14 days) 2% clinoptilolite within the colostrum, did not influence WBC.[17] In another study, prolonged clinoptilolite was used in cow ration at 1.25% and 2.5% levels and, denoted no effect on WBC.[13] In the present study, marked alteration (p<0.05) was detected in WBC values among groups in mid-lactation period. Period X group interaction revealed that there was a significance (p<0.05) regarding intergroup interaction. This significance was deemed in Zeolite I group in late lac tation. Significance in WBC count in Zeolite I group might be attributed to alimentary

TABLE 5: The least square means and standart variations of body condition score among different periods and trial groups.

Stages	Control N=10	Zeolite I (1.25%) N=10	Zeolite II (2.5%) N=10	p
Ration adaptation	$3.02^{a} \pm 0.141$	$3.10^{a} \pm 0.076$	$3.20^{a} \pm 0.133$	**
Early lactation	$3.45^{b} \pm 0.110$	$3.42^{b} \pm 0.112$	$3.55^{ab} \pm 0.116$	
Mid-lactation	$3.65^{bc} \pm 0.130$	$3.72^{c} \pm 0.114$	$3.65^{b} \pm 0.140$	
Late lactation	$4.00^{c} \pm 0.117$	$4.17^{d} \pm 0.091$	$4.19^{c} \pm 0.122$	

a,b,c: Means with different superscripts in each line are different; **: $p<0.01$.

tract irritation induced by zeolite particles, as reported previously.[17]

Conducting this search, the author was aware of finding documented reports regarding this subject, whereas it should not be unwise to make interpretation through prior data. A prior research involving 30 primiparous and multiparous lactating Holstein cows were classified to 3 different dietary treatments each involving 10 cows: control [Total Mixed Ration (TMR) diet], TMR diet with 1.4% $NaHCO_3$, and TMR diet added 1.4% zeolite. In that study milk yield was identical in both groups in which efficacy was not influenced.[18]

Apart from above mentioned studies with disperancy results, the authors' prior research involving 80 Holstein-Friesian cows between their first and third lactation, 4 months clinoptilolite addition resulted in increased milk production with mean values [33.66±0.756], in comparison to control cattle [30.63±0.851] ($p<0.01$).[8] Increased milk yield was also reported previously.[18] Furthermore 2% (w/v) clinoptilolite addition for 4 months markedly ($p<0.01$) influenced milk yield.[19] In the present research regarding daily milk yield, zeolite groups I and II revealed 2.38 kg and 3.29 kg increases, respectively, in comparison to the control group with statistical significance ($p<0.01$). The data purchased from the present research and of undelined research articles denoted that clinoptilolite addition might display marked efficiancy on milk production. These results were found to be consistent with another study.[20]

In this research, AST and ALT activities showed some significant variations. Previous literature denoted that highest AST activity was detected during early lactation, whereas during progression of lactation, the activity of AST decreased, which was also the case in this study.[21] The serum levels of AST were higher in pigs supplemented with clinoptilolite whereas still remained in the physiological levels.[22] Similarly, in this research the prominent AST analysis presented values within reference ranges.

ALT activity in cows differs in various production period. The lowest ALT activity might be detected in early lactation.[21] In the present study ALT levels presented the lowest activity in late lactation. ALT does not play a significant role for prediction of liver injury.[21] A previous study analyzed the effects of natural clinoptilolite in terms of hematological data and enzyme activities in sera (AST, ALT and LDH) in calves in the first three months of life. All 3 groups of calves were fed with standard feed in which the experimental groups received colostrum with additive (clinoptilolite as 5 and/or 10g/L milk).[15] In that study, regarding enzyme activity it was suggested that the examined preparation caused no marked alterations.[15] In comparison to the latter study, in this research ALT values presented variances between 24.40-28.60 U/L in ration adaptation, 27.70-36.80 U/L in early lactation, 18.80–21.90 U/L in mid-lactation and 18.60-23.30 U/L in late lactation. The differences between ALT values found to be significant in late lactation ($p<0.05$).

In a prior study analyzing long-term (iniated on 7 months of gestation through finishing of the first lactation period) clinoptilolite addition on milk yield and the energy status of dairy heifers, 80 Holstein pregnant heifers were classified into 2 groups: One with 0.2 kg clinoptilolite/day; and another (control) solely basal ration. In the latter study, clinoptilolite supplementation significantly elevated BCS, in which the researchers concluded that clinoptilolite supply might have helped improved performance.[20] In comparison with the previous study, BCS values were increased at the end of lactation in the present research. The differences among stages for each group were found to be significant ($p<0.01$). There has been limited studies regarding the relationship between zeolite (clinoptilolite) and BCS, in which a brief explanation for proposed mechanisms including animal's performance influencing characteristics of dietary usage for zeolites should be related to elimination of toxic effects of NH_4^+ as induced by gut microbial activity.[4]

In this research, one of the important indicator of the lipid profile, i.e. triglycerides measured on early lactation ($p<0.05$), and at the late lactation ($p<0.01$) presented significant alterations among control and Zeolite II groups, respectively. It may be briefly explained with the differences in lipomobilization due to the different stages of lactation and the effect of zeolite used at different levels in this study. Furthermore investigation of the influence of clinoptilolite supply revealed higher triglyceride concentration which was recorded in pigs receiving clinoptilolite.[22] Contrarily, serum triglyceride levels had lowering values throughout the study in Zeolite II group which received 2.5% zeolite. It is important to take into account that the influence on serum triglyceride levels is valuable and the possible mechanisms should be further studied.[22]

In conclusion, hematological and serum biochemical parameters indicated that dietary added zeolite did not cause side effects and long-term zeolite administration did not influence normal physiological homeostasis in animals.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

This study is entirely author's own work and no other author contribution.

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