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IMMUNITY RESPONSE AND REPRODUCTIVE PERFORMANCE OF LACTATING FRIESIAN COWS TREATED WITH BENTONITE

W.M. Wafa[1*], A.M. El-Hais[2], and Y.A.E. Yahia[2]

1- Animal Production Research Institute, Agricultural Research Center, Giza, Egypt, 2- Department of Animal Production, Faculty of Agriculture, Tanta University, Tanta, Egypt
[*]Corresponding Author's E-mail: drwailfatoh1973@hotmail.com

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SUMMARY

This study was conducted to determine the effect of dietary supplementation of bentonite on the immune status and reproductive performance of lactating Friesian cows. A total of 30 pregnant Friesian cows (4-6 years of age) were divided into three groups (10 cows in each). In the 1st group; G1, animals were fed the control diet, while those in the 2nd; G2 and 3rd; G3 groups were fed the control diet supplemented with 20 or 40 g bentonite/kg concentrate, respectively, from 60 days prepartum up to 120 days postpartum. Blood samples were taken at estrus and 120-d postpartum. Results show that calf weight at calving and placental drop time improved (P<0.05) in G3, while uterine horn symmetry and cervical closer improved in G2 and G3 compared with G1. Plasma immunoglobulins (IgG, IgM, and IgA) increased (P<0.05) in G3 than in G1 and G2. Count of RBCs and WBCs, hemoglobin, and PCV improved (P<0.05) by both treatments, being the highest (P<0.05) in G3. Interval to first estrus, service period, and days open were the best (P<0.05) in G3. Concentration of P_4 was the lowest at estrus, and the highest at 120-d postpartum in G3. Number of services/conception and pregnancy rate were 1.4 and 50% in G1, 1.6 and 80% in G2, and 1.4 and 100% in G3.

In conclusion, dietary supplementation of bentonite particularly 40 g/kg of concentrate to dairy cow from 60 days prepartum to 120 days postpartum may enhance their immunity and reproductive performances.

Keywords: bentonite, Friesian, hematology, immunoglobulins, reproduction

INTRODUCTION

Friesian cattle are raised in Egypt as one of the great agricultural constituents, and it is the main source of milk production. Increasing animal productivity is important for developing the sector of milk production to increase the income of smallholders (Mohamed *et al*., 2008) and obtain one calf/head/year.

The transition period (3 wks pre- to 3 wks post-partum) is considered as challenge for the lactating cows due to several physiological alterations occurred during this period, particularly early postpartum period (Grummer, 1995). As a result of increasing the nutrient requirements to milk production, and decreased dry matter intake the negative energy balance occurred (Mullins *et al*., 2012), and can cause hepatic lipidosis due to the excessive uptake from non-esterified fatty acids from adipose tissues by liver (Bobe *et al*., 2004), which impairs health, immunity, and reproductive efficiency of lactating cows (Burton *et al*., 2005).

To reduce the feed cost, and enhance milk production efficiency, it is important to enhance the dietary requirement benefits by using feed additives. Clay minerals such as bentonite are natural derived substances which can bind toxins by surrounding them in the layers of a multi layered structure, which makes a large surface area. The positive charge between the sheet layers of bentonite can negatively attract charged ions and elements (Spieker, 2010), and bind them. Bentonite has been used in animal diets as organic and inorganic adsorbent additives. Clay minerals contain different members like Tafla that obtained naturally from Egyptian mines it was used widely by commercial dairies as feed additives (bentonite) in ruminant diets to improve productivity, immunity, and reproductive performance (Salem et al., 2001). Bentonite is utilizing in agricultural applications, environmental remediation, pharmaceuticals, and many other industrial applications (Murray, 2007). In animal feeds, the calcium bentonite had been used in large scales because of the calcium ions presence that provide a better separation in clay layers which may improve the absorption of aflatoxin in ruminant rations (McClure *et al*., 2014). The positive impacts of bentonite on increasing milk production of dairy cattle were reported by Mikolaichik and Morozova (2009). Also, it had a beneficial effect on animal immunity especially in unusual conditions like feeding aflatoxin-contaminated diet (Abdel-Wahhab *et al*., 2002). Moreover, it can help in disposing of virus infection (Bellou *et al*., 2014) by binding with aflatoxin or virus rapidly and preferentially from digestive tract to reduce their absorption (Carraro *et al*., 2013). On the other hand, no evidence of a benefit of bentonite in feeding for adsorbing endotoxins in the ration in transition cows and it was suggested further research for evaluating the endotoxin binders efficacy on cow's health during the transition period and bentonite role in this concern (Razavi *et al*., 2019).

Based on the positive impacts of bentonite, the present study aimed to evaluate the effect of dietary supplementing different levels of bentonite on immunity status and reproductive performance of lactating Friesian cows.

MATERIALS AND METHODS

The present study was carried out at Animal Production Research Institute (APRI), Agriculture Research Center (ARC), Ministry of Agriculture in cooperation with the Department of Animal Production, Faculty of Agriculture, Tanta University, Egypt.

Animals and experimental groups:

A total number of 30 lactating Friesian cows (weighing 495 kg, aging 4-6 years, and within 2-4 parity) at 2 months prepartum were used in this study. All animals were fed according to NRC (2001) requirements on diet containing concentrate feed mixture, fresh berseem (2^{nd} cut, *Trifolium alexandrinum*), and rice straw. Cows were divided randomly into three groups according to their weight, parity, and milk yield (10 animals in each). Animals in the 1^{st} group (G1) were fed control diet without any treatment during the experimental period. While, in the 2^{nd} (G2) and 3^{rd} (G3) groups, animals were fed the control diet supplemented with 20 or 40 g bentonite/kg concentrate.

Feeding system:

The concentrate feed mixture (CFM) contained uncorticated cotton seed cake (65%), wheat bran (9%), rice polish (20%), molasses (3%), limestone (2%), and NaCl (1%). Chemical analysis, on dry matter basis, of feed stuffs of the control ration is presented in Table 1. Feeds were offered to animals at 8 a.m. and 4 p.m., while clean drinking water was available at all day time.

The supplemented bentonite was added as an Egyptian bentonite product (Bentonite OCMA®, Elwafaa For Trading & Contracting S.A.E, Egypt). It composed of 0.62% MgO, 56.30% SiO_2, 10.02% Fe_2O_3, 22.12% Al_2O_3, 1.32% TiO_2, 4.39% CaO, 1.13% $(Na)_2O$, 1.40% K_2O, and 10.75% LOI (Ismaeel *et al*., 2017). The determined amount of the supplemented bentonite was added to 500g of ground CFM immediately at the feeding time.

Table 1. Chemical analysis of different feedstuffs used in feeding cows of the experimental groups

Item	DM %	Chemical analysis on DM basis (%)					
		OM	CP	CF	EE	NFE	ASH
CFM	90.63	90.35	17.04	9.21	3.01	61.09	9.65
Rice straw	92.14	82.71	4.29	34.13	0.93	38.57	17.29
Berseem (2^{nd} cut)	13.62	86.03	17.54	24.12	2.29	42.08	13.97
Bentonite	92.91	7.03	-	7.03	-	-	92.97
Total ration	90.49	87.59	12.78	19.50	2.06	53.25	12.41

DM: Dry matter OM: Organic matter CP: Crude protein CF: Crude fiber EE: Ether extract NFE: Nitrogen free extract
CFM: Concentrate feed mixture

Milking:

After calving, the lactating cows in different experimental groups were milked twice daily at 6 a.m. and 4p.m. using a milking machine.

Blood samples:

Blood samples were taken from animals in each group at estrus, and at the end of the experimental period (120-d postpartum). Blood samples were collected from the jugular vein into dry and clean test tubes with heparin as anticoagulant, and then hematological parameters were determined. Thereafter, blood samples were centrifuged at 3500 rpm for 15 min to obtain plasma, which was stored at -20˚C until analysis.

In blood plasma taken at estrus and 120-d postpartum, concentration of progesterone (P_4) were determined according to Nulsen and Peluso (1992) by radioimmunoassay (RIA) using a commercial kit (DSL-3900 ACTIVE®). However, immunoglobulins (IgG, IgM, and IgA) concentrations were determined in blood plasma samples at the end of the experimental period using the quantitative ELISA (Bovine IgG, IgM, and IgA ELISA, Quantitative kit, Bethyl laboratories, UK) as described by Killingsworth and Savory (1972).

In the whole blood at the end of the experimental period, hematological parameters including the count of red and white blood cells (RBCs and WBCs), hemoglobin concentration (Hb), and packed cell volume (PCV%) were determined.

Reproductive performance:

Immediately after calving, body weight of newborn calves and the duration of placental drop were recorded. Also, the period elapsed from calving to symmetries in gravid uterine horn and the complete closure of the cervix was recorded.

During the postpartum period, all cows were observed for estrus and cows showing heat were artificially inseminated by fertile proven/thawed semen 12 h after the detection of estrus. Using the rectal palpation, animals were examined for observation of uterine involution. The interval from calving to first estrus, service period length, number of services per conception, and days open (d) were determined. Pregnancy was diagnosed on day 45-50 post-insemination, then number of services and conception rate was calculated for cows conceiving during the experimental period (120-d postpartum).

Statistical analysis:

Data were statistically analyzed by IBM SPSS (version 25, 2017) analysis program using one way-ANOVA. The significant differences among means were set at ($P<0.05$) using multiple rang test (Duncan, 1955). The following statistical model was used: $Y_{ij} = \mu + G_i + e_{ij}$

Where: Y_{ij} = Observations, μ = overall mean, G_i = The fixed effect of i^{th} treatment, where i =1,2,3 and e_{ij} = residual error.

RESULTS

Calving performance:

Results presented in Table 2 showed that live body weight of calves at birth increased by 16% ($P<0.05$) in G3 and in G2 by 8.5% as compared to G1. Duration of placental drop was shorter in G3 by 1.4 h ($P<0.05$) and in G2 by 0.95 h ($P\geq0.05$) compared with G1. The differences between G2 and G3 in LBW and placental drop duration were not significant. Cows in G2 and G3 showed early uterine involution in terms of reducing ($P<0.05$) the time elapsed from calving to horn symmetry and complete closure of the cervix as compared to G1, being with the best results in G3.

Table 2. Effect of bentonite supplementation on calving performance of lactating cows

Item	Treatment*			SEM	P- value
	G1	G2	G3		
Calf weight at birth (kg)	33.10^{b}	35.90^{ab}	38.40^{a}	1.71	0.006
Placental drop duration (h)	4.05^{a}	3.10^{ab}	2.65^{b}	0.23	0.038
Uterine horn symmetry (d)	41.80^{a}	33.60^{b}	30.60^{c}	1.01	0.000
Cervical closer (d)	46.20^{a}	36.50^{b}	33.30^{c}	1.15	0.000

a-c: Values with different superscripts within the same row are significantly different at ($P<0.05$).
* G1= control, G2= 20g bentonite/kg concentrate and G3= 40g bentonite/kg concentrate.

Immune status:

Plasma immunoglobulins:

Data presented in Table 3, revealed the highest concentrations of plasma immunoglobulins (IgG, IgM, and IgA) in G3 in comparison with other groups. Concentration of IgG, IgM, and IgA was higher ($P<0.05$) by about 23, 34, and 71%, respectively in G3 compared to G1.

Table 3. Effect of bentonite supplementation on concentration of immunoglobulins G, M, and A in lactating cows blood

Type of Ig (mg/ml)	Treatment*			SEM	P- value
	G1	G2	G3		
IgG	21.22^{b}	22.43^{b}	26.07^{a}	0.57	0.000
IgM	4.12^{b}	4.70^{ab}	5.52^{a}	0.23	0.025
IgA	0.52^{b}	0.61^{b}	0.89^{a}	0.05	0.001

a-b: Values with different superscripts within the same row are significantly different at ($P<0.05$).
* G1= control, G2= 20g bentonite/kg concentrate and G3= 40g bentonite/kg concentrate.

Hematological parameters:

Hematological parameters including count of RBCs and WBCs, hemoglobin concentration, and PCV % were higher ($P<0.05$) in G2 and G3 as compared to G1, being with maximal values in G3 (Table 4).

Table 4. Effect of bentonite supplementation on concentration of hematological parameters in lactating cows

Hematological parameter	Treatment*			SEM	P- value
	G1	G2	G3		
RBCs ($x10^6/mm^3$)	7.32^{c}	9.17^{b}	10.19^{a}	0.25	0.000
WBCs ($x10^3/mm^3$)	7.35^{b}	8.81^{a}	9.49^{a}	0.22	0.000
Hemoglobin (g/dl)	8.67^{c}	10.12^{b}	11.80^{a}	0.32	0.000
Packed cell volume (%)	31.40^{b}	34.10^{a}	34.30^{a}	0.53	0.035

a-c: Values with different superscripts within the same row are significantly different at ($P<0.05$).
* G1= control, G2= 20g bentonite/kg concentrate and G3= 40g bentonite/kg concentrate.

Postpartum reproductive performance:

Reproductive parameters including interval from calving to first estrus, service period length, and days open were shorter ($P<0.05$) in G3 than in G1 and G2 (Table 5). However, number of services per conception was nearly similar in all groups, being 1.4, 1.6, and 1.4 in G1, G2, and G3, respectively.

Table 5. Effect of bentonite supplementation on postpartum traits in lactating cows

Postpartum traits	Treatment*			SEM	P- value
	G1	G2	G3		
Postpartum 1st estrus interval (d)	68.2[a]	58.4[b]	44.8[c]	1.87	0.000
Service period (d)	29.0[a]	20.6[ab]	7.8[b]	3.05	0.045
Number of services/conception	1.4	1.6	1.4	0.08	0.112
Days open (d)	99.0[a]	80.6[a]	52.2[b]	5.35	0.000

a-b: Values with different superscripts within the same row are significantly different at ($P<0.05$).
* G1= control, G2= 20g bentonite/kg concentrate and G3= 40g bentonite/kg concentrate.

Plasma progesterone profile:

Plasma P_4 concentration at estrus recorded statistically the lowest ($P<0.05$) value in G3, followed by G2, and the highest in G1. Plasma P_4 concentration at 120-d postpartum showed an opposite trend, being higher in G3 than in G1 and G2 (Fig. 1).

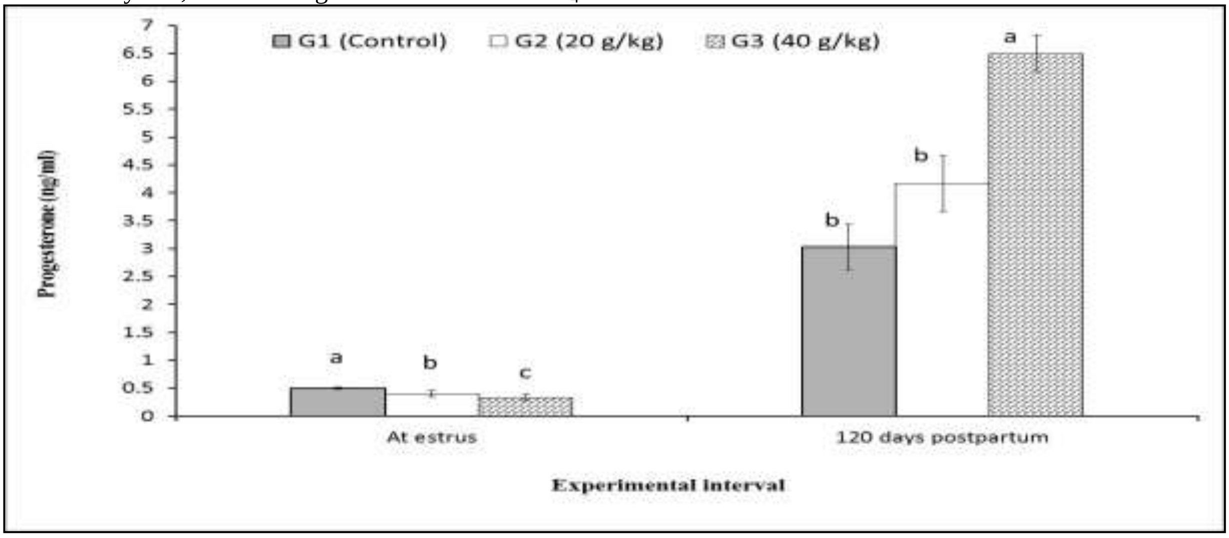

Fig. 1. Effect of bentonite supplementation on blood progesterone concentration at estrus and after 120-d postpartum.

Pregnancy rate:

Results of pregnancy rate (PR) during an interval of 120-days postpartum are illustrated in Fig. 2. These results indicated that following the 1st service, PR was higher (6/10, 60%) in G3 than in G1 and G2 (3/10, 30% for each). At the end of 120-d postpartum, all cows in G3 were conceived versus 80% (8/10) in G2, and the lowest PR value was obtained for cows in G1 (5/10, 50%).

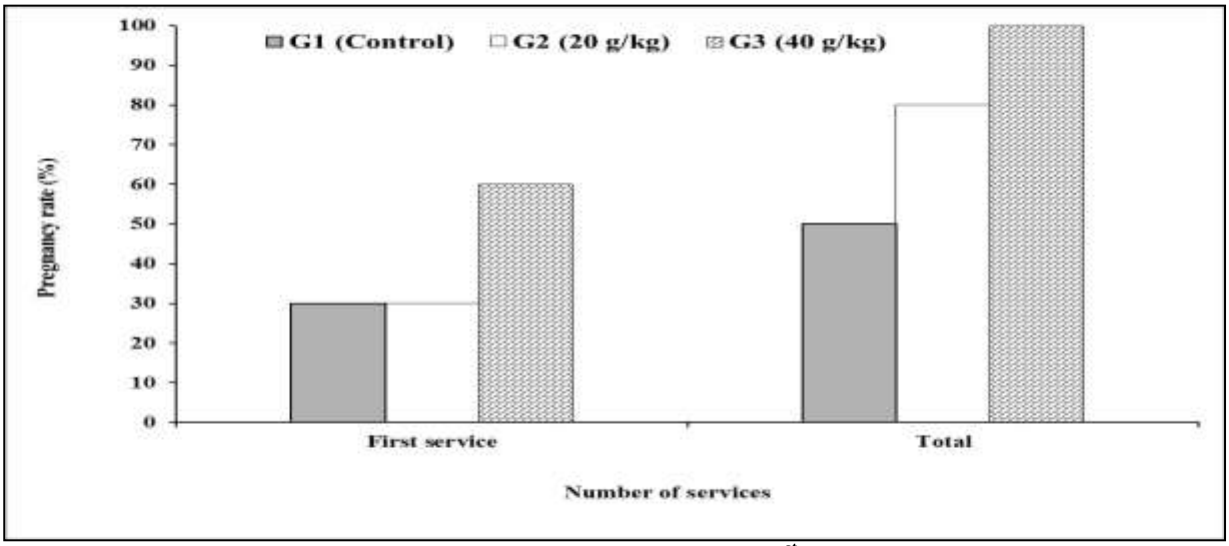

Fig. 2. Effect of bentonite supplementation on pregnancy rate from 1st and total number of services.
G1= control, G2= 20g bentonite/kg concentrate and G3= 40g bentonite/kg concentrate.

DISCUSSION

Usage of bentonite as feed additive showed beneficial effects on milk production (Mikolaichik and Morozova, 2009), immunity (Abdel-Wahhab *et al*., 2002), and as anti-virus infection (Bellou et al., 2014) in dairy cows. However, some authors had no evidence of bentonite benefits in the diet of cows and suggested further studies on endotoxin binders efficacy on cow's health in the transition period (Razavi et al., 2019). Therefore, the aim of the current study was to evaluate the effect of prepartum dietary supplementing bentonite (2 months prepartum) on calving performance as well as pre- and post-partum treatment (4 months postpartum) on immunity and reproductive performance of lactating Friesian cows.

The presented results showed significant effect of prepartum bentonite supplementation at a level of 40 g/kg diet on calving performance by producing calves with proper LBW at birth. This result reflects the beneficial effect of bentonite supplementation in improving the body condition and feed utilization of dietary nutrients in pregnant treated cows as compared to controls. These findings are in agreement with some improvement in Hanwoo steers body weight in association with bentonite supplementation (Young-Jik et al., 2017), and body weight of litter and sow fed diet with clay elements supplementation (Kyriakis et al., 2002). Contrary, Lee *et al*. (2010) did not find any effect for clay elements on the body weight gain of Hanwoo cattle. Results of calving performance also cleared marked effect of bentonite (40 g/kg) on reducing the duration of placental drop in association with reduced the interval to uterine involution that described by uterine horn symmetry and closer of the cervix. Improving the uterine involution as affected by bentonite administration was reflected in early resumption of postpartum ovarian activity (shortening interval to 1st estrus), and reducing service period length and days open. Several reports indicated that postpartum lactating cows with early uterine involution had improved reproductive efficiency of Friesian and buffalo cows (Abdel-Khalek *et al*. 2013, 2015). This improvement was in parallel with proper number of services per conception (1.4 services/conception). Pešev *et al*. (2011) recorded a decrease in the service period by about 36% in Simmental cows fed diet supplemented with zeolite as a factor in the improvement of reproductive traits of dairy cows. In our study, the reduction in service period length by bentonite treatment in G3 was higher, being 272% (from 29.0 to 7.8 day). In accordance with the present results, Karatzia *et al*. (2013) reported marked reduction in days to first estrus of Holstein heifers fed diet with clay elements (clinoptilolite). It worth noting that P_4 concentration decreased at estrus and increased at 120-day postpartum in cows treated with bentonite. The range of plasma P_4 concentration is within the normal range of Friesian cows in Egypt at estrus and 60 days post-service (Wafa, 2008). The P_4 profile observed at estrus may indicate higher pregnancy rate of cows in G3. Several authors indicated this finding (Abdel-Khalek et al., 2018; Abo-Farw *et al*., 2019). It is well known that P_4 showed an elevation during pregnancy, thus increment P_4 profile at 120-d postpartum in cows treated with bentonite (G3) was in relation with conceiving all cows in G3 versus 80 and 50% in G2 and G1, respectively.

The observed improvement in pregnancy rate as affected by the highest level of bentonite in G3 may be attributed to increasing immunoglobulin's concentration and elevating the hematological parameters. Values of studied hematological parameters in our study are within the normal range as described by Ogunade *et al*. (2016) for healthy dairy cows. In general, immune and hematological parameters had been affected by the dairy cattle nutrition plan (Radkowska and Herbut, 2014). Improving hematological parameters led to an improvement of the immune system of dairy cattle (Wafa et al., 2020). Bentonite has a role in limiting the bioavailability of aflatoxins through ion exchange (Moschini et al. 2008). The dietary supplementation of Holstein cows fed aflatoxin contaminated diets with clay elements could improve their production and immune status (Jiang *et al*., 2018). Immunity of lactating Holstein cows fed diet with mycotoxin can be improved by addition of clay elements via increased blood neutrophils cells, as the first defense line of the innate immune system (Ogunade *et al*., 2016). Anyway, clay elements can improve dairy cow immunity by binding with aflatoxins in the digestive tract (Diaz *et al*., 2004) that decreased its absorption in animal organs (Phillips et al., 2002).

CONCLUSION

In conclusion, the clay elements, in form of bentonite, as dietary supplement at a level of 40 g/kg during 2 months prepartum and 4 months postpartum could be used as a tool for improving the immunity and postpartum reproductive performance of lactating Friesian cows, which can improve the income of the small breeders, and relieve the milk shortage problem in Egypt.

Compliance with ethical standards:
Statement of Animal Rights:

This study was fulfilled under the standards of animal care that used for scientific purposes approved by the Ethics Committee of Tanta University, Egypt. We further followed the Directive 2010/63/EU recommendations for animal protection (Official Journal of the European Union, 2010) in animal's management.

Conflict of interest

The authors declare that, they have no interest conflict.

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الإستجابة المناعية والأداء التناسلي لأبقار الفريزيان الحلابة المعاملة بالبنتونيت

وائل محمد وفا[1]، عبدالعزيز محمد الحايس[2] ، يحي علاء الدين يحي[2]

1- -معهد بحوث الإنتاج الحيواني، مركز البحوث الزراعية، الجيزة،مصر، 2- قسم الإنتاج الحيواني، كلية الزراعة، جامعة طنطا، طنطا، مصر

أجريت هذه الدراسة لمعرفة تأثير إضافة البنتونيت على الحالة المناعية والأداء التناسلي لأبقار الفريزيان الحلابة حيث تم إختيار عدد 30 بقرة فريزيان عشار (4-6 سنوات) وتم تقسيمها إلى ثلاث مجموعات (10 أبقار في كل مجموعة). في المجموعة الأولى (G1) تم تغذية الحيوانات على عليقة ضابطة (كنترول) ، بينما تم تغذية الحيوانات في المجموعة الثانية (G2) و الثالثة (G3) على العليقة الضابطة مع إضافة 20 أو 40 جم من البنتونيت / كجم من العلف المركز من 60 يومًا قبل الولادة وحتى 120 يومًا بعد الولادة. تم سحب عينات الدم عند الشياع وعند 120 يوم بعد الولادة. أظهرت النتائج أن وزن العجل عند الولادة والفترة حتى نزول الأغشية الجنينية تحسنت معنويا (P<0.05) في المجموعة الثالثة (G3) ، بينما تحسن كل من الفترة حتى تماثل قرني الرحم و غلق عنق الرحم في المجموعة الثانية (G2) والثالثة (G3) مقارنة مع المجموعة الأولى (G1). حدثت زيادة معنوية (P<0.05) في الجلوبيولينات المناعية لبلازما الدم في المجموعة الثالثة (G3) عنها في المجموعة الأولى والثانية (G1 و G2). حدث تحسن معنوي (P<0.05) في عدد كرات الدم الحمراء وكرات الدم البيضاء والهيموجلوبين والـ PCV في كلا المعاملتين ، وكان الأعلى في المجموعة الثالثة (G3). كانت الفترة من الولادة حتى الشياع وفترة التلقيح والفترة حتى التلقيح المخصب هي الأفضل معنويا (P<0.05) في المجموعة الثالثة (G3). كان تركيز هرمون البروجيستيرون في أقل مستوياته عند الشياع ، والأعلى عند 120 يومًا بعد الولادة في المجموعة الثالثة (G3). كان عدد التلقيحات اللازمة للحمل ونسبة حدوث الحمل 1.4 و 50٪ في المجموعة الأولى (G1) و 1.6 و 80٪ في المجموعة الثانية (G2) و 1.4 و 100٪ في المجموعة الثالثة (G3).

الخلاصة إن استخدام البنتونيت وخاصة بمستوى 40 جم/ كجم من العلف المركز كإضافة غذائية للأبقار الحلابة خلال الفترة من 60 يوم قبل الولادة حتى 120 يوم بعد الولادة يُحسن المناعة والأداء التناسلي.