



Some Aspects of Using Clinoptilolite in Calves Feeding

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The use of clinoptilolite is an effective means of increasing the gain and live weight of calves. The application does not cause difficulties in the methods of introducing the additive to calves. Natural clinoptilolites of various deposits are presented in a wide range on the feed additives market.

Aims: The objective of the work was to study the effectiveness of natural mineral clinoptilolite on physiological and zootechnical indicators of growth and development of calves.

Methodology: The study was carried out on 39 clinically healthy Holsteinized black-and-white calves selected at random, 13 animals per group. For 85 days, the calves of the experimental group received a basic diet enriched with natural mineral clinoptilolite to increase the efficiency of growth and development at a dosage of 25-50 and 50-100 g / head / day. Blood samples were taken from the jugular vein at the end of the supplement feeding (n = 5) for physiological, biochemical and immunological studies.

Results: In the course of the study, it was found that in the experimental groups there were higher gains in live weight by 7.3 and 4.7% in comparison the control, and low feed costs per 1 kg of gain. Feeding clinoptilolite promoted an increase in the concentration of Ca in the blood of calves of the

dairy period by 14.2% ($p < 0.05$), an increase in phagocytic activity by 4.87 ($p < 0.05$), the phagocytic number was higher by 0.20 ($p < 0.05$) units.

Conclusion: The totality of the information presented confirms the physiological adequacy for calves to the introduction of natural mineral clinoptilolite in the indicated dosages.

Keywords: Calves; mycotoxins; zeolite calves' productivity; biochemical status; immunological status.

1. INTRODUCTION

Research on using natural zeolites as a biological feed additive for farm animals and poultry feeding started in the 60s of the last century remains relevant today. Interest in this mineral is not lost due to the unique physical and chemical structure and properties that allow, due to sorbing ion exchange, catalytic and other properties, to increase the animals' efficiency by optimizing the metabolic processes in their bodies.

In recent decades, more in-depth research has been performed to study a variety of natural zeolite – clinoptilolite. This subspecies was first described in 1969 by Owl Canyon [1]. This rock is widely known for its high acid-resistant silica content with Si/Al - 1/5 ratio, and large-scale worldwide deposits, where the clinoptilolite content varies from 50% and above. To date, this terminology is applied to samples with an unknown predominance of extra-framework cations, such as clinoptilolite-Ca, clinoptilolite-K, and clinoptilolite-Na [2].

The EFSA expert opinion (2013) on the safety of natural zeolite clinoptilolite *in vivo* has been published, which confirms its non-toxicity as a feed additive. According to EFSA, oral consumption of this zeolite type due to its extreme chemical stability poses no potential risk for *in vivo* use. The FEEDAP Panel concluded that 10,000 mg of clinoptilolite/kg of complete feed can be considered safe for feeding to all animal species. Clinoptilolite is practically not absorbed and excreted in the faeces. There is no evidence that clinoptilolite will decompose during its travel through the gastrointestinal tract of the target animals. Therefore, the consumer is not affected by clinoptilolite as a result of its use in the animal feed; therefore, there is no risk for the consumer [3].

The molecular clinoptilolite features, characterized by high adsorption properties and ion exchange, allow its use as an antiviral agent [4]. Due to the detox positive effect, which has no negative impact on the living organism, the

clinoptilolite *in vivo* use has been widely studied recently in veterinary science and medicine [5].

In recent years, the use of both natural and synthetic zeolites in animal feeding has increased, mainly to protect against mycotoxins and other xenobiotics. For the past years the fact that natural pollutants mostly affect the ruminants, since xenobiotics have a strong negative effect, primarily on the ruminal microflora has been quite proven [6,7,8].

The clinoptilolite supplement to the newborn calves' colostrum has a positive effect on the serum γ -globulin fractions and total protein concentration, wherein the dose of 0.5% rather than 2% gives the best results [6]. 5 g/kg of the clinoptilolite-based mineral adsorbent in the colostrum leads to a significantly higher degree of colostral IgG absorption in newborn calves. Also, clinoptilolite has no negative effect on the number of white blood cells, the metabolite concentrations in the blood and the enzymes activity. Taking clinoptilolite involves no serious side effects relating to the treatment [9,10].

Clinoptilolite supplementation can increase serum iron level, improve hematopoiesis, and prevent pathological or physiological decline in red blood cell parameters of vealers during the first few weeks of life [11,5]. Also, the clinoptilolite supplement to vealers' feed can affect the concentration of calcium, phosphorus, sodium and iron in the blood serum, but does not affect the concentration of potassium and magnesium. According to the higher Ca demand and use in growing animals, the clinoptilolite supplement can increase the available Ca [9,5,8].

Based on experience, the addition of 1.0 g clinoptilolite per kg of body weight per day to colostrum and milk is an appropriate dose to reduce the frequency and severity of diarrhea in newborn Holstein calves. Extending the use of clinoptilolite over 1.0 g / kg BW / day to colostrum and milk was considered excessive. More than 1.0 g / kg of body weight per day, clinoptilolite had a negative effect on passive immunity and

diarrhea. Because of its low cytotoxicity to intestinal cells and the results obtained *in vivo*, clinoptilolite may represent an alternative method of reducing the amount of antimicrobials required for symptomatic treatment of diarrhea in calves, thereby helping to reduce the phenomenon of antimicrobial resistance [9,12].

Thus, the issues of effective clinoptilolite use (different levels of feeding finely ground fraction) in the ruminant diets, in particular calves, are practically important for further study from the point of view of clinoptilolite effect on the animals' health and efficiency.

2. MATERIAL AND METHODS

The study was conducted on 39 clinically healthy Holstein calves of black-and-white breed (6 heifers and 7 steers in each group), randomly selected, 13 animals in the group. The calves were selected at the age of one month with an initial live weight of 46 kg. The duration of the experiment is 85 days.

The natural mineral sorbent used in the experiment had a particle size of <0.20 mm and contained: 56% of clinoptilolite, mordenite – 5-8%, quartz – 2.5-4%, the admixture of opal-cristobalite – 32% (SiO₂·nH₂O), which was determined by X-ray powder diffraction. Chemical composition, %: SiO₂ – 73.2; Al₂O₃ – 11.9; Fe₂O₃ – 1.16; CaO – 2.09; MgO – 1.12; TiO₂ – 0.08; K₂O – 3.23; Na₂O – 0.55; MnO – 0.03.

In energy and nutritional indexes the young cattle diet met the detailed rates of feeding young cattle (R.V. Nekrasov *et al.*, 2018).

The housing conditions for all groups of calves (temperature, humidity, light regimes and gas composition of the indoor air) were the same and within the limits of zoohygenic standards.

During the scientific experimentation using weaners, they were weighed at the beginning of

the animals testing, on the 54th and 85th day of the accounting period.

To determine the effect of different clinoptilolite rates on the forage consumption, an individual accounting of forages and their residues was carried out every decade throughout the entire accounting period. At the end of the experiment, the feed was studied by determining the forage consumption per unit of the resulting products.

The mycotoxin content was determined by the multi-method Spectrum HPLC-MS/MS (High-performance liquid chromatography-mass spectrometry (HPLC-MS/MS); Agilent 1290/AB SCIEX Triple Quad 5500; GOST 34140-2017) in samples of forages, which form the animal diet.

Blood samples from calves (N=13, n=5) were taken from the jugular vein at the end of the experiment; blood was collected from each animal before morning feeding in vacuum glass test tubes. After coagulation, serum was separated by low-speed centrifugation, transferred to plastic vials and sent for biochemical analysis.

The concentrations of calcium (Ca), phosphorus (P), magnesium (Mg), total bilirubin (BIL), cholesterol (Chol), glucose (Glu), triglycerides (TG), total protein (TP) and the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined in serum by the standard protocol using the automatic biochemical analyzer Chem Well (Awareness Technology, USA) in the Department of Physiology and Biochemistry of L.K. Ernst Federal Research Center for Animal Husbandry.

In the Department of Microbiology of L.K. Ernst Federal Research Center for Animal Husbandry, the indicators of nonspecific resistance were determined in samples of calves' whole blood and serum (N=13, n=5).

Table 1. Experimental design of experiment

Groups	Number animals	Animals age, day of growing	
		31-84	85-116
		The way of supplement feeding, g/head/day	
		with milk	with mixed feed
1 Control (C)	13	-	-
2 Experimental (E1)	13	25	50
3 Experimental (E2)	13	50	100

Table 2. Composition and nutritional value of the average vealers' daily diet during the experiment

Name	Content, kg
Milk	1,900
The whole milk substitute	1,900
Cereal hay	1,100
Perennial grass haylage	1,000
Prestarter	0,120
Starter	1,200
Table salt	0,011
Phosphate	0,015
Natural mineral sorbent	a)
Total	5,500
Nutritional value of the feeding diet (% by DM)	
metabolic energy for cattle, MJ	33,9
dry matter, kg	2,9
crude protein, g	399,0
digestible protein, g	280,8
crude fat, g	166,9
crude fibre, g	463,8
nitrogen-free extractives, g	1628,6
starch, g	444,3
sugar, g	321,5
Ca, g	20,5
P, g	14,0
S, g	5,8
Mg, g	5,1
NaCl, g	16,0
Additional diet supplementation with nitrogen-free extractives, no less than	
vitamin A, thousand IU	100,0
vitamin D, thousand IU	20,0
vitamin E, mg	20,0
Cu, mg	50,0
Fe, mg	150,0
Zn, mg	250,0
Mn, mg	100,0
Co, mg	5,0
I, mg	5,0
Se, mg	2,0

a) – according to the experimental design

Bactericidal activity (BA) of blood serum was measured by turbidimetric method in a spectrophotometer UNICO-2100 (United products & instruments, Ins. USA) at OD540. E. coli culture (0.005 ml) was mixed with 4.5 ml of Tryptic Soy Broth (TSB) (Merck, Germany) and 0.5 ml of blood serum in sterile tubes. Control was 0.5 ml of physiological saline with phosphate

buffer instead of serum. All tubes were cultured at 37°C for 5 h.

Percentage of BA was calculated from the following formula:

$$\%BA = ((Dk - Do) / Dk) * 100$$

Dk is optical density of control;

Do is optical density of experimental sample.

Lysozyme was measured by turbidimetric method in a spectrophotometer UNICO-2100 (United products & instruments, Ins. USA) at OD540. Tubes of blood serum (0.1 ml) were heated (56°C) in a water bath for 30 minutes, then 1.4 ml of standard *M. luteus* culture was added and incubated at 37°C for 3 h. The following parameters were determined: lysozyme activity of blood serum (LA), concentration of serum lysozyme (lysozyme, µg/mL), activity unit (AU) per 1 mg protein (AU/TP).

Lysozyme activity (LA) of a blood serum is calculated using the following formula:

$$\%LA = ((\Delta Do) * 100 / Do1) - ((\Delta Dk) * 100 / Dk1)$$

Do is the difference in the optical density of the prototype,

Dk is the difference in the optical density of the control,

Do1 is the optical density of the prototype immediately,

Dk1 is the optical density of the control.

The concentration of lysozyme in serum was calculated based on calibration with dilutions of chicken egg-white lysozyme (L6876, Sigma) ranging from 0.1 to 51.2 µg/ml.

Due to variations in protein content in the blood serum of animals, the level of lysozyme activity was converted and expressed in arbitrary units of activity per 1 mg of protein (activity units per 1 mg of TP or AU/TP).

The phagocytic activity of blood cells was evaluated by determining the absorption and digestion capacity of blood cells. The phagocytic capacity of blood leukocytes was judged by the data of their phagocytic activity, indicators of total phagocytic content, phagocytic number and

index, as well as the indicator of completed phagocytosis.

The groups were formed randomly, based on the physiological state of the calf when examined by a veterinarian and the body weight. Statistical data processing was carried out by the method of variational statistics using Student test on a PC using the software package STATISTICA (version 10, StatSoft, Russia).

3. RESULTS AND DISCUSSION

It was found that specific forage types (mainly concentrates) contained average and high level of certain mycotoxin types. Thus, in corn grain, there was an excess of type A trichocetenes MPC, moderate contamination with type B trichocetenes; soya beans – moderate contamination with type B trichocetenes; barley, oats – moderate contamination with type B trichocetenes. It is worth noting *Alternaria* fungal mycotoxins (not rated in the Russian Federation) in some feeds (for example, cakes, extruded soya bean), which enhance the effect of the main mycotoxins. Bulk feed meets the standards for all studied mycotoxins. Thus, it is necessary to evaluate the feed background as satisfactory in terms of contamination with different mycotoxins.

Different rates of feeding natural mineral sorbent affected the vealers' growth indexes (Table 3). Animals that consumed 25 and 50 g/head/day of clinoptilolite had higher live weight gains than the control, and low feed costs per 1 kg of gain.

Biochemistry showed that the blood metabolic profile in all groups was within the physiological range. This indicates that the studies were performed using clinically healthy animals (Table 4).

Table 3. Calvers' growth dynamics during the experiment (M±m, n=13)

Index	Group		
	C	E1	E2
Live weight, kg:			
- at the age of 31 days	46.60±1.49	46.22±1.96	46.33±3.07
- at the age of 84 days	94.67±2.93	96.96±2.72	95.41±3.39
- at the age of 116 days	113.90±3.09	118.44±2.98	116.78 ±4.78
Average daily gain, g	791.76±39.61	849.67±32.52	828.76±27.07
Feed costs per 1 kg gain, MJ ME	42.8	39.9	40.9

Table 4. Biochemical blood parameters (M±m, n=5)

Parameter	Group		
	C	E1	E2
Total protein, g/L	76.31±2.30	77.63±1.04	77.47±4.35
Albumin, g/L	25.59±1.53	28.42±0.82	29.38±1.10
Globulin, g/L	50.72±3.06	49.21±1.60	48.09±3.75
A/G ratio	0.51±0.06	0.58±0.03	0.62±0.04
Urea, mmol/L	7.58±0.44	7.40±0.47	6.64±0.38
Alkaline phosphatase, IU/L	559.38±166.61	490.76±102.34	657.81±141.69
Glucose, mmol/L	7.62±1.27	8.47±1.20	7.65±0.74
Calcium, mmol/L	2.39±0.14	2.57±0.04	2.73±0.04*
Phosphorus, mmol/L	3.15±0.33	3.26±0.024	3.40±0.15
Ca/P	1.01±0.08	1.04±0.09	1.05±0.04
Creatinine, µmol/L	50.51±5.77	56.74±4.32	64.11±7.46
Bilirubin, µmol/L	19.05±2.89	10.14±0.91*	10.66±2.19*

Significantly at * - $p < 0.05$ compared to the control

Table 5. Resistance index of experimental animals (M±m, n=5)

Index	Group		
	C	E1	E2
Lysis, %	10.62±0.86	13.13±1.31	11.87±1.31
Serum lysozyme, mcg/ml	0.23±0.01	0.26±0.02	0.24±0.02
Sp.act.u. act. U/mg of protein	0.77±0.06	0.90±0.08	0.83±0.07
BA, %	33.55±3.71	27.74±2.93	30.32±3.53
PA, %	30.40±1.35	35.27±1.10*	34.83±1.46
PI	1.51±0.12	1.43±0.14	1.91±0.13
PN	0.46±0.05	0.51±0.06	0.67±0.06*

Significantly at * - $p < 0.05$ compared to the control.

During the study, the initial biochemical parameters of the blood were not studied. The calves of the experimental and control groups were in the same conditions, and comparisons were made relative to the control group. Calves for the study was selected randomly, and in a quantity that allows us to assess the reliability of the sample.

The level of total bilirubin increases in animals with hemolytic jaundice, increased hemolysis of red blood cells. The content of bilirubin in the blood should be 1-14 µmol/L. Feeding clinoptilolite helped to reduce its level in the blood of calves. At the end of the experiment the blood serum of vealers treated with different natural mineral sorbent rates had 53.22 and 55.96% ($p < 0.05$) less bilirubin concentration compared to the control.

The calcium content in the blood serum of vealers treated with 50 and 100 g/head/day increased by 14.22% ($p < 0.05$) compared to the control. However, there were no significant differences between the groups in phosphorus, Ca/P content. The clinoptilolite applied did not

produce any clinically visible disorders in the metabolism of the tested minerals. In addition, the blood levels of all tested minerals were within physiological ranges, which indicates that clinoptilolite did not alter their homeostasis in calves [13].

Feeding the natural mineral sorbent had a significant effect on the phagocytic activity index. In addition, feeding the sorbent positively affected the phagocytic number and a higher rate of sorbent contributed to increasing the number of phagocytes involved in phagocytosis (Table 5).

The main aim of the experiments was to determine the efficiency of using finely-ground (<0.2 mm) clinoptilolite fraction in diets of vealers on metabolism and immune status of young cattle when feeding traditional feeds. Main mycotoxin content was found in the concentrated feed. Even low rates of several mycotoxins can lead to clinical and negative effects on the immune system under stress or during infectious diseases. As a result, the antibody level, vaccination efficacy, phagocytes,

immunoglobulins and lymphocytes activity decrease the animal productivity [14]. It should be noted that the feed contamination with several mycotoxins leads to their simultaneous entry into the body, depending on their absorption rate, mycotoxins have a synergistic or additional effect, DON increases the fumonisins adsorption and zearalenone increases the negative effect of DON [3].

The diet supplementation with different rates of finely-ground natural sorbent had a positive effect on the intensity of protein metabolism in vealers. This confirms the fact that high rates of clinoptilolite supplement to mycotoxin-containing diets of calves has no adverse effect on productivity and contributes to normalize the protein metabolism by improving the pre-gastric digestion in animals. Alic Ural & Ural [15] showed that the addition of the clinoptilolite via colostrum appeared to enhance ADG in newborn calves without having any observable adverse effect.

Research performed by Katsoulos et al. [16] to study the effect of clinoptilolite feeding on the productivity and biochemistry of dairy goats' blood serum; experiments with clinoptilolite supplements to cows and vealers' feed [17,18] show that prolonged feeding the natural mineral sorbent as part of the concentrated forages does not negatively affect the productivity and metabolic parameters in the animal body, positively affecting homeostasis and biochemistry of protein and mineral metabolism. In a review by Pavelić et al. [8] it is indicated that the additional supplements of 3% clinoptilolite-based feed additive to the diet of growing young pigs by researchers contributed to an increase in nitrogen excretion in the feces and a decrease in nitrogen excretion in the urine of animals. This is why clinoptilolite has been widely used in animal husbandry for many years as an additive to animal feed or to remove ammonia from biological media and animal manure. Both in the experimental groups on rearing young cattle and in the scientific experiment on cows, we noted a tendency to increase the total protein in the blood serum compared to the control, which, most probably, is related to more intensive protein metabolism due to the high ion exchange capacity of the natural mineral sorbent and effective feed toxicity prevention. The positive effect of the diet supplementation with zeolites was confirmed in many studies on young animals with an increase in live weight gain and feed conversion in pigs, calves, cows and sheep. One of the earlier studies performed by Pourliotis et

al. [18], where clinoptilolite at a dose of 1 and 2 g/kg of body weight per day with colostrum and milk was used as a feed additive for vealers and indicates a faster growth of experimental animals compared to the control group, increased intestinal antibody absorption vs. enterotoxigenic *Escherichia coli* strains, as well as reduced the frequency and duration of diarrhea in vealers. Pelin & Adem [19] concluded that the use of clinoptilolite, which is used in many different areas, in calf diarrhea will shorten the treatment period.

It is known that insufficient mineral nutrition causes a deep disorder in the metabolism, reduces the body resistance, contributing to diseases. During our experiment the rich composition of the mineral elements of clinoptilolite contributed to an increase in Ca concentration in the vealers' blood ($p < 0.05$). The research results indicate that clinoptilolite feeding positively affected Ca and P level in the vealers' blood, improving their ratio at the end of the experiment. This is also seen in Folnožić et al. [17]. The results of this study suggest that dietary clinoptilolite influenced the blood levels of Ca and P in dairy cows, and improved the serum Ca:P ratio of dairy cows during the early postpartum period. Clinoptilolite did not cause any clinically visible disturbances in the metabolism of the studied mineral elements in the animal body.

The use of clinoptilolite in animal feeding prevents the animals' morbidity, strengthening their immunity. Studies performed by Gradzki et al. [20] showed that clinoptilolite has an effect on the increase in the resistance of young poultry to infection, which is confirmed by the results of clinical observations and anatomical and pathological studies on rearing broiler chickens, as well as by increasing the synthesis of acute-phase proteins with immunoregulatory properties. The results of these immunological studies indicate that 2% zeolite supplement as a feed additive is most effective for prolonged homeostasis in chickens. The authors indicate that 3% clinoptilolite induces the production of anti-inflammatory cytokines, increasing the interferon synthesis, which enhances the humoral immune response while inhibiting the cytokines production. In Đuričić et al. [21] cows treated with clinoptilolite (50 g/head/day) had a 1.96-fold lower risk of intramammary infection than the control cows. Our studies also prove that clinoptilolite affects the increase in resistance of young cattle, which is confirmed by an increase in phagocytic activity ($p < 0.05$) and

clinical observations of the vealer condition during the experiment. The use of clinoptilolite, therefore, can be used in particular when replacing antibiotic-containing preparations, as well as in combination with medications, to improve protection against infections, improve digestive processes and health of calves [22, 23].

4. CONCLUSION

The main conclusion of these studies that using of clinoptilolite allows to timely prevent a number of non-specific diseases related to the mycotoxins intake, lack or excess of trace elements in feed, improve the animals' health, increase the body's defenses, thereby contributing to an increase in the intensity of their growth with a significant reduction in feed costs per unit of production, and thereby obtain environmentally friendly livestock products.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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