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# EFFECTS OF ZEOLITE SUPPLEMENTATION ON DAIRY COW PRODUCTION AND RUMINAL PARAMETERS – A REVIEW

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**Abstract**

**In recent years, the use of both natural and synthetic zeolites in livestock feeds fed to lactating cows has increased, mainly to improve their performance, health, and to protect against mycotoxins intoxication. Data of scientific literature were compiled to analyze the effects of the incorporation of non-nutritional adsorbent zeolite on production performance and ruminal environment parameters of lactating cows. At moderate levels (200–400 g/cow/day), milk yield was increased by zeolite. Milk fat and protein contents and DMI were not altered and all ruminal parameters were improved: acetate was enhanced, propionate was reduced and consequently, acetate to propionate ratio was increased. The rumen pH was increased and rumen ammonia nitrogen was reduced. When the level of zeolite exceeded 400 g/d/cow, all production and ruminal parameters were negatively altered. These data suggest that zeolite level in the diet has a significant effect on the response of dairy production and ruminal environment characteristics.**

**Key words: dairy cows, lactation, productive parameters, ruminal parameters, zeolite**

In recent years, the increase in the genetic potential of dairy cows has led to the massive use of concentrates in the ration in order to meet their needs. Unfortunately, this practice has caused several problems: milk fat depression, lower fiber digestibility, and increased herd health problems related to acid-base disturbances (Davis, 1979; Muller and Kilmer, 1979; Snyder et al., 1983).

In order to alleviate or prevent metabolic disorders that are associated with the consumption of high concentrate diets by dairy cows, the inclusion of dietary buffers has become a common and accepted practice. The most popular one is sodium bicarbonate ($NaHCO_3$), but other buffers like sodium carbonate ($Na_2HCO_3$), di-sodium phosphate ($Na_2PO_4$), potassium carbonate ($K_2CO_3$) and potassium bicarbonate ($KHCO_3$) were also used. Their use was well-documented and resulted in several research studies and reviews (Hu and Murphy, 2005; Iwaniuk and Erdman, 2015).

With the inclusion of these buffers in high concentrate and low fiber diets, milk fat can be maintained at normal concentrations. The improvements had been attributed to high ruminal pH and osmolality, which enhance ruminal fermentation and increase ruminal outflow. Supplementation with buffers also decreases propionate production in the rumen and increases the acetate:propionate ratio, which improves the milk fat test (Davis, 1979).

Although these mineral additives have received widespread usage, their inclusion into the diet is costly for the producer (Eickelberger et al., 1985; Harrison et al., 1986; Rogers et al., 1985). Therefore, a series of experiments have been conducted to identify cheaper mineral buffers that exhibit the same mode of action as the established buffers. Examples of such minerals are both natural (clinoptilolite) and synthetic zeolites (zeolite A).

Zeolites are crystals formed from a microporous aluminosilicate skeleton of alkali and alkaline earth cations which are encountered worldwide and having an infinite, open, three-dimensional structure. These materials have unique chemical and physical properties and are characterized by their ability to lose and gain water reversibly, to absorb substances with a suitable cross-sectional diameter (adsorption property) and to switch their cations with cations from their environment such as $K^+$, $NH_4^+$, $Ca^{2+}$, and $Mg^{2+}$, without major structural change (cation exchange capacity-CEC ≈ 220 meq/100 g) (Bosi et al., 2002; Filippidis et al., 1996).

The facility of these supplements to liberate progressively ions in the rumen has created in the past 20 years an interest to use them as feed additives for ruminants, mainly in order to improve performance traits and for the prevention of certain metabolic diseases in dairy cattle. Another reason to use zeolites in the feed is to reduce the release of ammonia in the manure (Dschaak et al., 2010; Ilić et al., 2011). Recently, zeolite has been approved as an additive in farm animal feeds by the European Committee at the highest inclusion rate of 2% of dry matter (European Commission Regulation, 2005). The effectiveness of clinoptilolite against intoxication by mycotoxins, as well as the increased interest for the use of feed additives that do not have residuals on the animal products, are expected to increase the use of clinoptilolite as a feed additive.

Numerous studies have been conducted with lactating dairy cows supplemented with zeolite, but with conflicting results (Fukushima, 1979; Johnson et al., 1988). For that, several original researches have been compiled and data are processed using high-performance statistical methods such as meta-analysis and principal component analysis (PCA) to assess the efficacy of supplemental zeolite on feed intake, milk production and composition, and ruminal fermentation characteristics when added to the diet of lactating dairy cows. Thus, the purpose of this synthesis document was to elucidate in what degree zeolite supplementation affects the dairy cows performances.

**Data sources**

From 1983 to 2016, research papers with appropriate study designs and with lactational production data containing between 14 and 42 comparisons were used. These studies are mainly originated from the USA and Europe and are obtained from

ScienceDirect and Journal of Dairy Science online databases using the combination of search terms “zeolite”, “clinoptilolite” and “dairy cows”. The paper was retained when key criteria between zeolite treatment and control groups were given. Articles were inspected for 2 major inclusion criteria: (1) studies should be *in vivo* involving lactating dairy cows, and (2) availability of information on zeolite level, diet composition (DM, CP, NDF, net energy), milk yield and composition, feed intake (DMI), days in milk (DIM), and ruminal parameters.

All studies in the database included zeolite in total mixed rations (TMR) for lactating cows. In all of them, diets consisted of a basal TMR as the control diet, and the basal TMR plus supplemental zeolite as the dietary treatments. Treatment and control diets were fed for *ad libitum* consumption. In the studies that included more than one supplemental buffer source in the TMR, only the treatment with zeolite was compared to control.

Stage of lactation was labelled as the media between the beginning and the end of the study. The 7 lactational parameters used in this study were milk yield (MY), milk fat content (MFC), milk protein content (MPC), milk fat yield (MFY), milk protein yield (MPY), 4% fat corrected milk (4%FCM) and dry matter intake (DMI). The 6 ruminal criteria were total volatile fatty acid (VFA), total volatile fatty acid (TVFA), acetate ($C_2$), propionate ($C_3$), butyrate ($C_4$), ammonia ($NH_3$) and pH. They were expressed as the difference from supplemental zeolite to control treatments. If certain parameters were not reported (for example 4% FCM), they were estimated and included in the analysis. The complete list of research papers used for this study was given in Table 1.

Table 1. List of the studies included in the analysis data set.

| References | References | References |
|---|---|---|
| Bergero et al. (1997) | Garcia Lopez et al. (1992) | McCollum and Galyean (1983) |
| Bosi et al. (2002) | Ghorbani et al. (1989) | Migliorati et al. (2007) |
| Butsjak and Butsjak (2014) | Grabherr et al. (2009 a) | Moate et al. (1985) |
| Cassida et al. (1988) | Hamilton et al. (1988) | Moore et al. (1992) |
| Clark et al. (2009) | Harrison et al. (1989) | Solorzano et al. (1989) |
| Đoković et al. (2011) | Ilić et al. (2005) | Sulzberg et al. (2016) |
| Dschaak et al. (2010) | Ilić et al. (2011) | Tucker et al. (1994) |
| Ehrlich and Davison (1997) | Johnson et al. (1988) | Ural (2014) |
| Enemark et al. (2003) | Karatzia et al. (2011) | Ural et al. (2013) |
| Erasmus and Prinsloo (1989) | Karatzia et al. (2013) | Vicentin et al. (1995) |
| Galyean and Chabot (1981) | Katsoulos et al. (2006) | |

**Statistical analysis**

The database was treated with SPSS software (Version 17.0). The first analysis took into account only the effect of zeolite dose to explain the response of each variable. In order to improve model precision, other variables such as lactation stage, NDF, CP and NE of supplemented diet, which seemed to explain a significant part of response variations, were integrated. Pearson correlation coefficients between variables, and their significance, were computed. Several types of regressions were generated (linear, quadratic…) and only the most significant ($P<0.05$) with the highest coefficient of determination ($R^2$) were retained.

Additionally, principal component analysis (PCA) was performed to quantify the overall production and ruminal parameters responses offered by the full dataset variation. PCA is a dimension-reduction procedure for multivariate data. For the lactational performance and ruminal characteristics data in this study, PCA summarized many components into a few scores (i.e. PC). Each PC may show a specific feature of variable data such as milk yield or DMI according to how the PCA adds the components in each PC. Thus, the purpose of applying PCA is to investigate changes in lactational and ruminal data using summarized components. The Bartlett's test of sphericity, which contains a P-value and a $\chi^2$ statistic, was carried out to confirm the viability of the PCA test in this review. We also calculated the contribution of each component to each PC by dividing the loading by the standard deviation of the component, because the PCA was based on the correlation matrix.

## Results and discussion

Descriptions and summary statistics of relevant measured dietary and animal variables are provided in Table 2. Significant correlations between studied parameters when zeolite was supplemented to dairy cows relative to controls were resumed in Figure 1. Responses to zeolite were highly heterogeneous for all variables studied.

Table 2. Overall summary statistics of studied parameters

| Parameters | Mean | SEM | Minimum | Maximum |
|---|---|---|---|---|
| DIM | 127.18 | 64.81 | 28 | 280 |
| Zeolite (g/cow/d) | 251.68 | 127.84 | 90 | 662 |
| CP (% of DM) | 15.68 | 2.72 | 10.14 | 19 |
| NDF (% of DM) | 32.71 | 5.49 | 26.9 | 45.21 |
| NEL (Mcal/kg DM) | 1.65 | 0.16 | 1.4 | 1.83 |
| ΔDMI (kg/d) | 0.33 | 0.84 | –2.1 | 1.8 |
| ΔMY (kg/d) | 1.02 | 1.71 | –2.7 | 4.7 |
| ΔFCM (kg/d) | 1.26 | 1.78 | –2.5 | 5.87 |
| ΔMFC (%) | 0.11 | 0.14 | –0.29 | 0.49 |
| ΔMPC (%) | –0.03 | 0.10 | –0.26 | 0.16 |
| ΔMFY (g/d) | 68.14 | 90.11 | –80 | 290 |
| ΔMPY (g/d) | 20.70 | 54.46 | –110 | 176 |
| ΔRumen (pH) | 0.09 | 0.14 | –0.18 | 0.47 |
| ΔTVFA (mM) | –0.41 | 9.92 | –18.3 | 24.03 |
| ΔAcetate (%) | 0.88 | 2.31 | –3.9 | 6.67 |
| ΔPropionate (%) | –0.77 | 2.07 | –5.9 | 3 |
| ΔButyrate (%) | 0.02 | 1.49 | –2.3 | 4.95 |
| ΔAcetate:Propionate | 2.80 | 0.96 | 1.36 | 4.68 |
| ΔRumen NH3 (mmol/l) | –0.30 | 1.05 | –2.5 | 1.5 |

Δ: Difference between zeolite treatment and control groups.

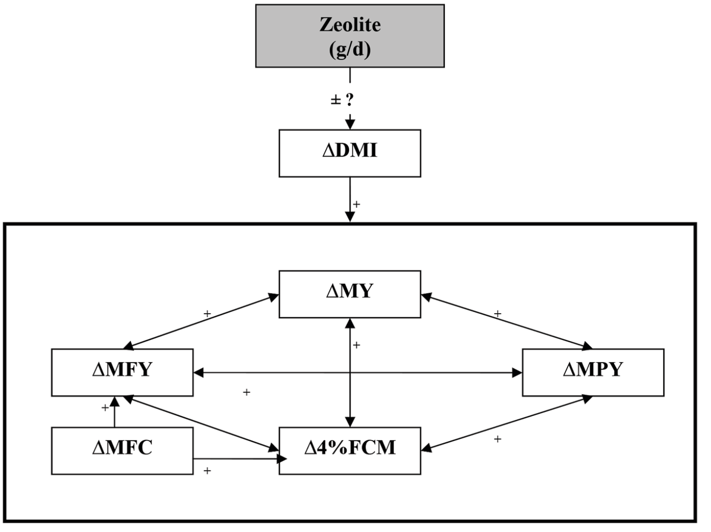


Figure 1. Significant correlations between studied productive parameters when zeolite was supplemented to dairy cows relative to controls

**Productive parameters**

*Feed intake*

A quadratic response was observed for feed intake by zeolite supplementation (Figure 2). Intake of DM by dairy cows was not altered at a moderate level (<300 g/d/cow). This trend was reversed and a rapid reduction of feed intake was detected at a high dosage of zeolite (>400 g/d/cow).

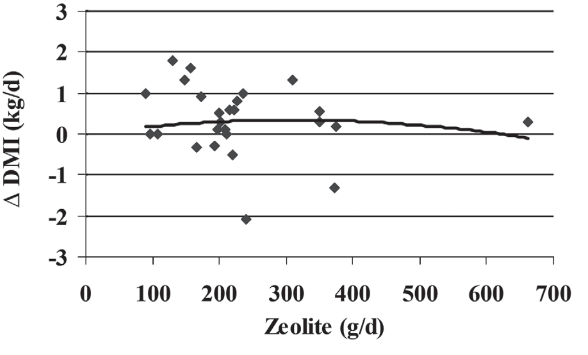


Figure 2. The response in dry matter intake (DMI) of dairy cows to the inclusion of zeolite relative to controls

Effects of buffer supplementation on DMI have been inconsistent, with buffer supplementation reported to decrease (Johnson et al., 1988; Thilsing-Hansen et al., 2002), not affect (Clark et al., 2009; Erasmus and Pinsloo, 1989; Grabherr et al., 2009 a; Sulzberger et al., 2016), or increase (Davis and Clark, 1983; Solorzano et al., 1989) DMI of dairy cows. These differences may be due to various sources or purities of used buffers.

The reason for the depressed feed intake is still not exactly clear. According to Carter and Grovum (1990), the negative effects of buffer diet on DMI can result partly because of the poor palatability of this additive (acceptability), and from increased ruminal osmolality. Moreover and according to Kincaid et al. (1981) and Thilsing-Hansen et al. (2007), decreases in DMI in cows fed high buffers were associated with a reduction in plasma P concentration (hypophosphatemia). A consequence of the reduced feed intake of cows was an insufficient supply of nutrients and energy. A negative energy balance as a consequence of less DM intake (DMI) leads to an increased lipolysis which enhances the risk of lipomobilization syndrome postpartum (Grabherr et al., 2009 a).

Positive responses for DMI are most often observed for cows fed diets that are low in effective fiber or when cows were fed diets supplemented with sodium bicarbonate that contained corn silage as the primary source of forage (Erdman et al., 1980,1982; Kilmer et al., 1981; Rogers et al., 1985). However, when alfalfa silage was included as a major part of the dietary forage, DMI was not altered in many experiments when sodium bicarbonate was added to the diet (Boisclair et al., 1986; Donker and Marx, 1980, 1985; English et al., 1983).

*Milk yield*

The response of milk yield to zeolite supplementation was dose-dependent (Figure 3). The mean milk yield tended to increase when zeolite was added to the diet at a moderate level (<300 g/d/cow). But this trend was reversed when zeolite addition exceeded 400 g/d/cow.

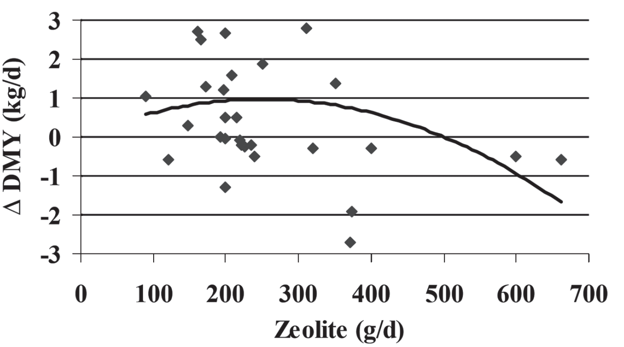


Figure 3. The response in milk yield (MY) of dairy cows to the inclusion of zeolite relative to controls

There is contradictory evidence about the effect of adding zeolites to the rations of dairy cows on their milk yield. Differences in results might be related to the supplementation level, to the composition of the diets, DMI, buffering capacity of the diet and its ability to stimulate saliva secretion, particle size of the diet and the dietary cation-anion difference DCAD (Davis and Clark, 1983; Hu et al., 2007; Iwaniuk and Erdman, 2015).

*Positive cases*

Many researchers have proved that the dietary inclusion of clinoptilolite or sodium bicarbonate improves average yield of dairy cows (Cruywagen et al., 2015; Ilić et al., 2011; Katsoulos et al., 2006; Ural et al., 2013). Generally, an increase in milk production can result from increased ruminal concentrations of propionate, increased postruminal digestion of starch, increased microbial protein synthesis, increased bypass protein, or from a combination of these factors (Garcia-Lopez et al., 1992; Katsoulos et al., 2006).

Other authors suggested that cows supplemented with buffers were able to convert feed into milk more efficiently (Cassida et al., 1988; Sulzberger et al., 2016; Ural, 2014). The results obtained from the present study and aforementioned researchers indicated that the administration of clinoptilolite improves the energy status of the animals and may have positive influence on milk yield (Karatzia et al., 2013; Katsoulos et al., 2006).

Another explanation for increases in production of milk might be the potential effect of zeolite on the DCAD of diets fed to the cows. Previous experiments (Hu et al., 2007) indicate that buffers increased DCAD and that DCAD and production by dairy cows are related. It appears that the DCAD of the diet affects acid-base balance regulation, which in turn increased DMI of dairy cows; this might explain a part of the increase in milk production.

*Negative cases*

Lower milk performance associated with the administration of a synthetic zeolite was reported by a number of authors (Johnson et al., 1988; Vetyška, 1996). According to Bosi et al. (2002) and Johnson et al. (1988), this reduction was likely associated with the decrease in DM intake and digestibility. This finding was confirmed in our study by the high correlation between milk yield and DMI ($r=0.59$, $P<0.005$; Figure 1).

*No effect*

In certain studies, the milk yield was not affected by zeolite inclusion in the diet (Grabherr et al., 2009 a; Katsoulos et al., 2006; Migliorati et al., 2007; Thilsing-Hansen et al., 2002) although the cows showed a reduced feed intake and hypophosphataemia. It appears that at low level of zeolite inclusion (1–1.4% on a DM basis), milk yield was not affected.

*Ration type*

Positive responses for yields of milk were reported when cows were fed diets supplemented with $NaHCO_3$ that contained corn silage as the primary source of forage (Erdman et al., 1980, 1982; Kilmer et al., 1981; Rogers et al., 1985). However, when alfalfa silage was included as a major part of the dietary forage, production of milk was not altered in many experiments when $NaHCO_3$ was added to the diet (Boisclair et al., 1986; Donker and Marx, 1980, 1985; English et al., 1983).

Addition of NaSC or bentonite to the diet did not affect milk yield when the higher forage diet (roughage, silage, pasture) was fed to the cows (Clark et al., 2009;

Hamilton et al., 1988; Moate et al., 1985). These data agree with the findings of Tucker et al. (1994) when diets were formulated to provide sufficient fiber of adequate particle size. In contrast, experiments with dairy cattle fed in feedlots have shown that bentonite increased milk yield when cows were fed high concentrate, low roughage rations (Rindsig and Schultz, 1970; Rindsig et al., 1969). To date, the addition of buffer to the diet has resulted in positive responses in productivity mostly when diets contain large amounts of concentrate and small amounts of fiber (Erdman, 1988; Hu and Murphy, 2005).

*Lactation stage*

Given that zeolites improve the energy status of the dairy cattle, especially in early lactation (Katsoulos et al., 2006), the dietary inclusion of this buffer might have beneficial effects on the productive performance of dairy cattle. However, a number of studies indicated that buffer supplementation had no influence on milk yield in lactating cows during early or midlactation (Cassida et al., 1988; Ghorbani et al., 1989; Solorzano et al., 1989). But enhancement in milk yield during late lactation was signaled by different authors (Clark et al., 2009; Erasmus and Pinsloo, 1989; Harrison et al., 1986). It was not known that the exact mechanism of buffer increased milk production at the end of lactation (Clark et al., 2009).

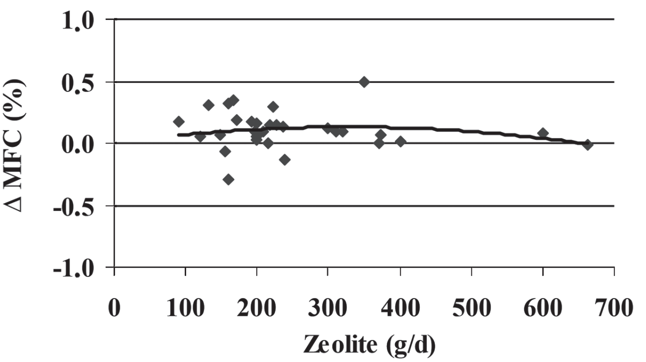


Figure 4. The response in milk fat content (MFC) of dairy cows to the inclusion of zeolite relative to controls

*Milk fat content*

In our study, MFC was not affected by zeolite at any level of supplementation (Figure 4). These data are in agreement with results of other experiments using different sources of buffers (Clark et al., 2009; Jordan and Aguilar, 1985; Migliorati et al., 2007). In contrast to our findings, other researchers reported that MFC increased or tended to increase when 1 to 2% of sodium sesquicarbonate or clinoptilolite was added to the diet (Cruywagen et al., 2015; Ghorbani et al., 1989; Solorzano et al., 1989; Sulzberger et al., 2016). Ruminal buffers have been shown to prevent milk fat depression with rations based on corn silage or low-fiber diets (Harrison et al., 1989; Kennelly et al., 1999) by stabilizing rumen pH and thus offering a more suitable environment for microbial growth. Additionally, the buffer may increase ruminal outflow, increasing the acetate: propionate ratio and improving milk fat tests (Cruywagen et al., 2015; Davis, 1979; Erdman et al., 1982; Snyder et al., 1983). Earlier reports from Rindsig et al. (1969) concluded that cows fed clay at 5% had increased acetate and decreased propionate in the rumen, leading to significant increases in milk fat percentage. According to Davis (1979), there is a negative correlation between milk fat concentration and molar proportion of rumen propionate when propionate exceeds 25%.

*Lactation stage*

Buffer supplementation had no effect on milk fat content in early or midlactation cows, but fat content was generally higher during late lactation (Tucker et al., 1994). Others (Cassida et al., 1988; Erdman, 1988; Ghorbani et al., 1989; Solorzano et al., 1989) reported that buffer supplementation increased milk fat percentage in cows in early or midlactation for diets based on corn silage or higher in concentrates.

*Ration type*

In North America, experiments with dairy cattle fed in feedlots have shown that bentonite increased milk fat concentrate when cows were fed high concentrate, low roughage rations or corn silage as the primary source of forage (Erdman et al., 1982; Kilmer et al., 1981; Rogers et al., 1985). In contrast, in studies with cows grazing pasture or when alfalfa silage was included as a major part of the dietary forage, milk fat was not altered in many experiments when sodium bicarbonate was added to the diet (Boisclair et al., 1986; Donker and Marx, 1985; Hamilton et al., 1988; Moate et al., 1985).

*Milk protein content*

In general, it has been accepted that dietary buffers do not consistently alter protein percentage of milk (Cassida et al., 1988; Coppock et al., 1986; Ghorbani et al., 1989; Solorzano et al., 1989) during early or midlactation. This trend was confirmed in our study (Figure 5), suggesting that protein metabolism was unaffected by the addition of clinoptilolite (Katsoulos et al., 2006).

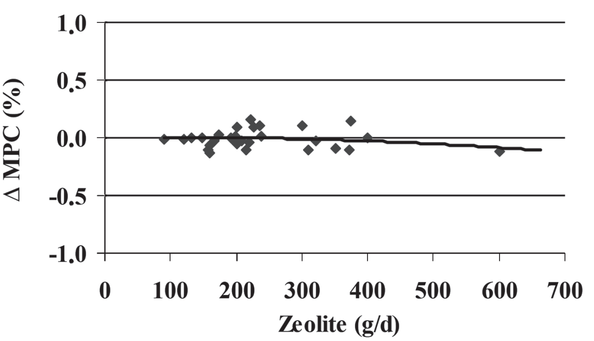


Figure 5. The response in milk protein content (MFC) of dairy cows to the inclusion of zeolite relative to controls

However, Dschaak et al. (2010) and Tucker et al. (1994) reported that milk protein percentage was increased with zeolite supplementation during the complete lactation. This difference did not appear until midlactation and was most apparent during late lactation. Solorzano et al. (1989) noted that sodium sesquicarbonate increased digestibilities of CP and NDF. Addition of buffer may have increased AA supply to the mammary gland by promoting digestion of dietary proteins.

*Milk fat yield, milk protein yield and 4% FCM*

Milk yield, milk protein yield (MPY), milk fat yield (MFY) and 4% fat corrected milk (4% FCM) were positively inter-correlated. MFY and 4% FCM were also positively correlated with MFC (Figure 1). Thus MFY and 4% FCM were enhanced only when MY and/or MFC were increased by zeolite addition. According to Cassida et al. (1988), Davis and Clark (1983) and Muller and Kilmer (1979), daily milk fat yield and 4% FCM yield were improved due to sodium bicarbonate or sodium sesquicarbonate supplementation, as a result of the improved milk fat percentage. But in other studies, milk fat yield was unaffected by buffer supplementation (Solorzano et al., 1989; Tucker et al., 1994).

Our data neither refute nor support previous data comparing the efficacy of sodium bicarbonate or sodium sesquicarbonate to increase fat-corrected milk, since no treatment effects on 4% FCM were detected with zeolite supplementation.

Thomas et al. (1984) reported that supplementation of sodium bicarbonate, MgO, or $Mg(OH)_2$ decreased milk protein content and milk protein yield relative to an unbuffered diet, contrary to Solorzano et al. (1989) who reported that milk protein yield was higher for cows fed sodium sesquicarbonate than for control. But other researchers (Cassida et al., 1988; Clark et al., 2009; Ghorbani et al., 1989; Tucker et al., 1994) did not detect buffer effects on milk protein yield.

**Ruminal parameters**

*Rumen pH*

The massive inclusion of readily fermentable carbohydrate feedstuffs in dairy rations causes the appearance of digestive disorders such as subacute ruminal acidosis if appropriate precautions are not taken. Strategic use of dietary ruminal buffers has been suggested as an alternative approach to avoid the occurrence of ruminal acidosis. Commonly dietary buffer addition has been used to prevent abrupt declines in rumen pH, which has been observed to be higher in cows fed buffers than in cows fed the same diet without buffers (Clark et al., 2009; Katsoulos et al., 2013). This chemical feed additive is characterized by an acid dissociation constant that is close to the normal ruminal pH. The high affinity of zeolites for water and osmotically active cations may facilitate ruminal fermentation, and osmotic activity may regulate pH in the rumen by buffering against hydrogen ions of organic acids (Dschaak et al., 2010).

In our study, a quadratic response for ruminal pH was observed consecutively to zeolite supplementation for dairy cows. At moderate level (<400g/day/cow), ruminal pH was increased. This trend was reversed when the supplementation level exceeded 500 g/day/cow (Figure 6). The raising in rumen pH following the use of various buffers was mentioned in numerous studies with synthetic zeolite (Dschaak et al., 2010; Johnson et al., 1988; Karatzia et al., 2011; Vicentin et al., 1995), with sodium bicarbonate (Davis, 1979; Erdman et al., 1982; Kilmer et al., 1981; Rogers et al., 1982) or with sodium sesquicarbonate supplementation (Jimenez, 1985). This increase is attributed to the buffer effects of clinoptilolite when added to acidic or basic aqueous solutions (Fillippidis et al., 1996). In the meta-analysis by Hu and Murphy (2005), rumen pH increased when buffered diets were used compared with unbuffered diets. The higher contents of magnesium and aluminum silicate may have contributed to the buffering capacity of the zeolite. Zeolite has been shown to work as alkalinizers and have a great capacity for $H^+$ exchange at different pH ranges (Yong et al., 1990). The authors reported that illite clay (a type of clay with high concentrations of magnesium and aluminum silicate) had the best buffer capacity in the pH range from 4.5 to 6, similar to the rumen pH range.

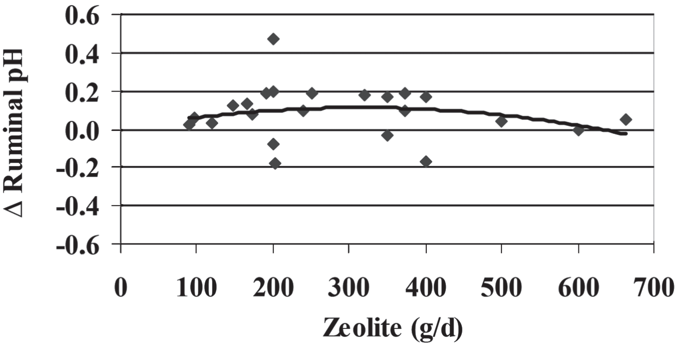


Figure 6. The response in rumen pH of dairy cows to the inclusion of zeolite relative to controls

Thus, buffers appear to modify the ruminal environment by maintaining pH within an optimal range (Davis, 1979; Okeke et al., 1983). Marden et al. (2008) suggested that sodium bicarbonate may have stabilized the pH through its strong capacity to neutralize protons.

Other researchers found that clinoptilolite does not affect ruminal pH (Bergero et al., 1997; Bosi et al., 2002; Grabherr et al., 2009 b; Johnson et al., 1988), whereas others have not reported any increase with sodium bicarbonate (Cassida et al., 1986; Erdman et al., 1980) or sodium sesquicarbonate (Cassida et al., 1986; Jordan and Aguilar, 1985). On the other hand, Galindo et al. (1986) observed a reduction of the same rumen parameter.

*Total volatile fatty acids*

The effect of zeolite on rumen TVFA was presented in Figure 7. At moderate level (400 g/cow/day), the concentration of the total fatty acids was not affected, but exceeding this level, this parameter was decreased. In some studies, the clinoptilolite supplemented diets tended to produce higher levels of TVFA due to increased liquid dilution rate (Davis, 1979; Rogers et al., 1982; Snyder et al., 1983), but in others, the concentration of the total fatty acids was reduced. This was the result of the faster removal of soluble substrates before they could be fermented (Erasmus and Prinsloo, 1989; Erdman et al., 1982; Vicentin et al., 1995).

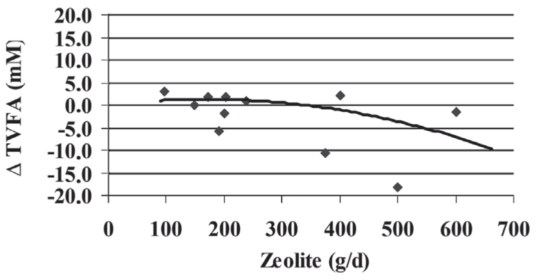


Figure 7. The response in rumen total volatile fatty acids (TVFA) of dairy cows to the inclusion of zeolite relative to controls

*Proportions of volatile fatty acids*

The addition of zeolite resulted in the highest rumen molar percentage of acetate and reduced propionate. This also resulted in a higher acetate:propionate ratio (Figures 8 and 9). This trend was also observed in numerous studies and with different buffers (Coppock et al., 1986; Erasmus and Prinsloo, 1989; Grabherr et al., 2009 b; Hu and Murphy, 2005; Johnson et al, 1988; Karatzia et al., 2011; Vicentin et al., 1995). Increases in acetate:propionate are frequently associated with increased rumen pH and digestion and due to a more favorable environment for the rumen microbial fermentation (Esdale and Satter, 1972; Johnson et al., 1988). Other authors associated the increase in molar percentage of acetate and the decrease in molar percentage of propionate in the rumen with the increased rumen liquid dilution rate (Davis, 1979; Erasmus and Prinsloo, 1989; Rogers et al., 1982) with the buffer additive. Such alterations on the rumen volatile fatty acid proportions could affect the energy status of the animals and cause changes to the milk yield and composition of dairy cattle (Karatzia et al., 2013).

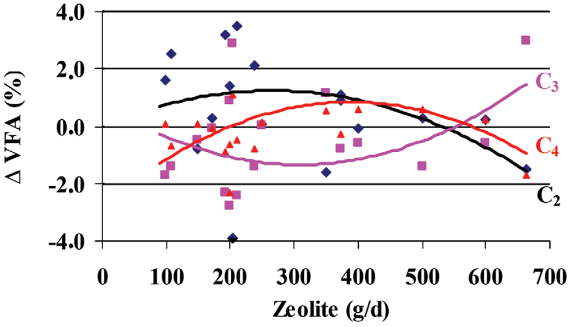


Figure 8. The response in rumen acetate ($C_2$), propionate ($C_3$) and butyrate ($C_4$) of dairy cows to the inclusion of zeolite relative to controls

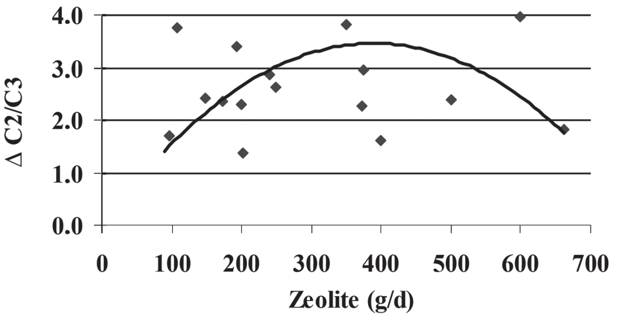


Figure 9. The response in rumen acetate ($C_2$) to propionate ($C_3$) ratio of dairy cows to the inclusion of zeolite relative to controls

In some studies, it was observed that zeolites increase the proportion of propionate (Galyean and Chabot, 1981; McCollum and Galyean, 1983) when clinoptilolite was added to high-concentrate diets and not at high-roughage diets.

No significant influences of zeolite A supplementation were observed for the molar proportions of butyrate (Grabherr et al., 2009 b; Karatzia et al., 2011). In the study of Ghorbani et al. (1989), the molar percentage of butyrate was even reduced in cows fed added sodium sesquicarbonate or sodium bicarbonate. This could mean that clinoptilolite did not adsorb this volatile fatty acid. But in our study, butyrate increased with zeolite to reach an optimal level at 500 g/day/cow (Figure 8).

*Rumen ammonia $NH_3$*

In this study, the rumen ammonia ($NH_3$) concentration was reduced when the level of zeolite incorporation increased (Figure 10), and may reflect absorption of ammonia by this additive.

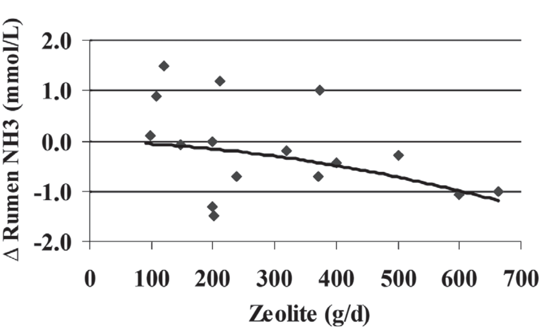


Figure 10. The response in rumen ammonia ($NH_3$) of dairy cows to the inclusion of zeolite relative to controls

In addition, supplementing zeolite in dairy diets may improve nitrogen utilization, because zeolite gradually releases excess ammonia in the rumen and allows rumen microorganisms to capture the $NH_3$ into microbial protein for assimilation into the animals' digestive systems (Mumpton, 1999).

Published effects of buffers on ruminal $NH_3$ concentration include increases (Grabherr et al., 2009 b; Kilmer et al., 1981), decreases (Erasmus and Prinsloo, 1989; Rindsig and Schultz, 1970), or no effect (Bosi et al., 2002; Eickelberger et al., 1985; Johnson et al., 1988; Vicentin et al., 1995). Finally, it is of interest to note the similarity between rumen pH and ammonia patterns.

*Principal component analysis for productive parameters*

Bartlett's sphericity test has an associated P value < 0.001 (sig. in Table 3). So from the above results, we know that we can now continue and perform a valid factor analysis. An important assumption in the principal component analysis relates to the effect of systematic factors on the correlations between lactational parameters. It improves understanding of the responses to zeolite supplementation.

Table 3. KMO and Bartlett's test eigenvalues, the proportion of variation described and loadings for principal components (PC) generated by the principal component analysis for productive parameters

| | | |
|---|---|---|
| KMO measure of sampling adequacy | **0.641** | |
| Bartlett's test of sphericity: | | |
| Approx. chi-square | 119.812 | |
| df | 21 | |
| Sig. | **0.000** | |
| **Principal component** | **PC1** | **PC2** |
| Standardized eigenvalues | 3.95 | 1.24 |
| Proportion of standardized variation | 0.54 | 0.20 |
| Loadings: | | |
| ΔMY | **0.91** | –0.17 |
| ΔMFC | 0.36 | **0.72** |
| ΔMPC | –0.10 | **–0.83** |
| ΔMFY | **0.89** | 0.25 |
| ΔMPY | **0.87** | 0.16 |
| ΔFCM | **0.97** | 0.12 |
| ΔDMI | **0.61** | –0.09 |

Δ: Difference between zeolite treatment and control groups.

The first two principal components had eigenvalues greater than one (Table 3). Together, they explained 74% of the total variance in the adjusted values of the studied parameters: PC1 accounting for 54% and the second principal component (PC2) for 20%. The loadings also given in Table 3 are simple correlations between the original and the new variables. A loading whose absolute value was greater than 50% (0.5) was considered to indicate that an original variable is influential in forming the new variable.

PC1 and PC2 from this analysis are shown in Figure 1. The position of each variable in the loading plot describes its relationship to the other variables. Variables which are close have high correlations. Variables on the same side of the origin (0.0) are positively correlated and those on the opposite side of origin are negatively correlated. When looking at just the loadings of the dependent variables on the biplot of PC1 versus PC2, two groupings of terms are observed.

The first grouping is seen in the upper right quadrant and includes MY, MPY, 4% FCM, MFY and DMI. All of these terms were closely related to each other and concerned the lactational yields. This is consistent with the regression analysis previously explained (Figure 1).

The second grouping of terms is seen in the centre of the two quadrants and includes MFC and MPC. These terms relate to the milk composition.

The PC1 had a relatively large influence on MY, MFY, MPY and 4% FCM and medium influence on DMI. It did indeed reflect the main consequences of net energy intake that limits yields of both milk and milk components when zeolite was supplemented.

PC2 was considerably correlated with MFC and MPC and was more difficult to interpret. In our review, the MPC was only positively correlated with CP level of the diet ($r = 0.60$, $P = 0.002$, data not shown). Another possible explanation could be that, to some extent, the dilution effect of milk yield was more pronounced on MPC that MFC when zeolite was incorporated in the diet. This further implied that MPC and MFC could not be easily described by the information provided by the dataset. But it was obvious that the factors driving milk and milk component yields were quite different from those affecting MFC and MPC when zeolite was included.

For the DMI and examining the loading plot in more detail, a high DMI seems to have been partially associated with the MY group (group 1, Figure 11). This implies that there is little probability of finding many supplemented rations with zeolite with both high DMI and high MY and milk component yields.

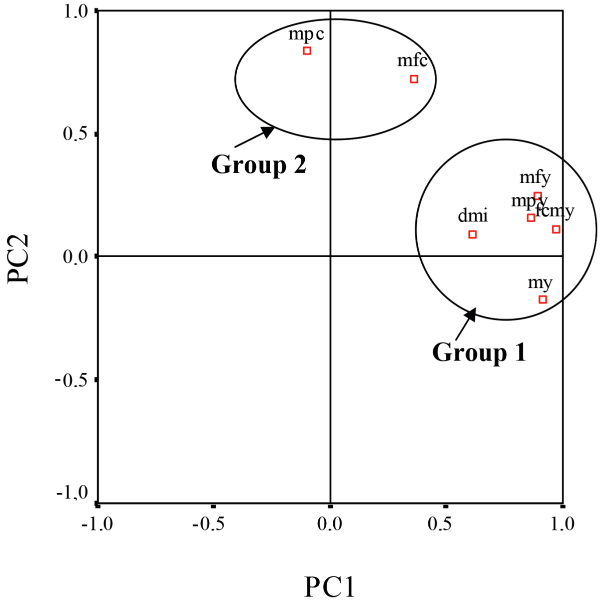


Figure 11. Component plot in rotated space for the productive variables obtained by PCA analysis

In summary, these results can be considered as a dashboard and can be exploited according to the objectives of the breeder during the incorporation of zeolite in the diet of dairy cows. For example, if the main objective concerns the improvement of milk production, this can be achieved when supplementation by zeolite was followed by an increase in DMI. This increase in milk production is accompanied by an increase in milk component yields. In addition, when MPC was enhanced, MFC was also increased (group 2, Figure 11).

*Principal component analysis for ruminal parameters*

The second PCA analysis was performed for ruminal parameters. Bartlett's sphericity test as an associated P value < 0.001 (sig. in Table 4). The first two principal components had eigenvalues greater than one (Table 4). Together, they explained 65% of the total variance in the adjusted values of the studied parameters: PC1 accounting for 42% and the second principal component (PC2) for 23%.

Table 4. KMO and Bartlett's test eigenvalues, the proportion of variation described and loadings for principal components (PC) generated by the principal component analysis for rumen parameters

| | | |
|---|---|---|
| KMO measure of sampling adequacy | **0.474** | |
| Bartlett's test of sphericity: | | |
| Approx. chi-square | 17.357 | |
| df | 21 | |
| Sig. | **0.000** | |
| **Principal component** | **PC1** | **PC2** |
| Standardized eigenvalues | 3.04 | 1.51 |
| Proportion of standardized variation | 0.42 | 0.23 |
| Loadings: | | |
| ΔAcetate | **0.87** | 0.31 |
| ΔRumen pH | **0.83** | 0.11 |
| $\Delta C_2:C_3$ ratio | **0.75** | 0.06 |
| ΔRumen $NH_3$ | **0.70** | 0.04 |
| ΔPropionate | –0.40 | **–0.89** |
| ΔButyrate | –0.47 | **0.81** |
| ΔTVFA | –0.26 | –0.28 |

Δ: Difference between zeolite treatment and control groups.

PC1 and PC2 from this analysis are shown in Figure 12. When looking at just the loadings of the dependent variables on the biplot of PC1 versus PC2, three groupings of terms are observed.

The first grouping includes ruminal acetate, pH, $C_2/C_3$ ratio and rumen $NH_3$. All of these terms were closely related to each other and concerned the rumen environmental conditions.

The second grouping of terms is seen in the centre of the two quadrants and includes ruminal butyrate and propionate. Thus, PC2 could be described primarily as a contrast between butyrate on one hand and propionate on the other.

Finally, the third grouping is in the lower left quadrant and consists of only the TVFA.

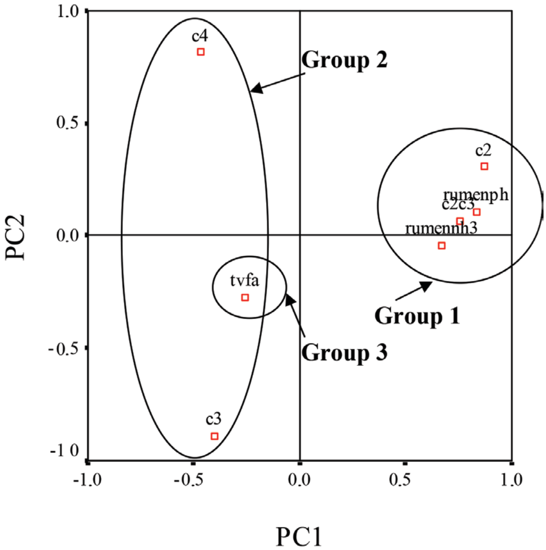


Figure 12. Component plot in rotated space for the rumen parameters obtained by PCA analysis

The PC1 had a relatively large influence on ruminal acetate, pH, $C_2/C_3$ ratio and $NH_3$ and medium influence on TVFA. It did indeed reflect the main consequences of buffer addition that promote yields of both rumen acetate and ammonia and enhanced rumen pH and $C_2/C_3$ ratio. In other terms, zeolite supplementation can ameliorate the ruminal environment and reduce the incidence of milk fat depression caused by feeding low roughage diets (Sutton et al., 2003).

PC2 was considerably correlated with rumen propionate and butyrate in opposite way. This negative relation between those two major VFA is well known (Sutton et al., 2003). In our review, the TVFA was the less affected parameter by zeolite supplementation. But when increased, propionate was enhanced more than butyrate (Figure 12). Thus the increase in propionate can be related directly to fermentable carbohydrate in the ration. This further implied that rumen butyrate and propionate could not be easily described by the information provided by the dataset. But it was obvious that the factors driving rumen acetate, pH and $NH_3$ parameters were quite different from those affecting rumen butyrate and propionate when zeolite was included.

For the TVFA and examining the loading plot in more detail, a high TVFA seems to have been partially associated with the butyrate and propionate group. This implies that there is little probability of finding many zeolite supplemented rations with both high TVFA and high rumen butyrate and propionate percentages.

In summary, the PC1 can reflect the rumen comfort by the parameters of group 1, normally obtained with high roughage diet (high NDF) and PC2 can reflect the effect of high fermentable carbohydrate diet on the rumen parameters (group 2) when zeolite was added to the ration of dairy cattle.

## Conclusion

The present study confirms that zeolite supplementation in dairy cows at moderate level was associated with an increase in DMI, milk yield, 4% FCM, and components in milk. This increase was brought about by enhancing ruminal pH and acetate/propionate ratio and by reducing ruminal ammonia level. We postulated that zeolite administration would alleviate the effects of a grain challenge and might affect the rumen or intestinal environment or modulate the acid-base metabolism. But at the high level, detrimental effects on milk production, milk quality, and ruminal environment are expected.

We concluded that non-nutritional adsorbent zeolite does not negatively affect productive and ruminal parameters when inclusion level does not exceed 400 g/cow/day. At this level, zeolite proved to be an effective buffer for the improvement of productivity in dairy herds.

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