

# Effect of Dietary Buffers Supplementation on Milk Yield and Composition in Dairy Cows: A Meta-Analysis of Randomized Controlled Trials

Meta Analysis

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## ABSTRACT

Feeding high concentrates to high producing animals usually change the rumen environment and compromises the productivity of ruminants. Different feed additives are used to prevent the occurrence of sub-acute ruminal acidosis, among these additives, buffers are commonly used. The aim of this meta-analysis was to investigate the effects of buffer supplementation on milk yield and composition in dairy cows. PubMed, Scopus, Web of Science and Google Scholar databases were searched from 1969 to 2020. A total of 86, 91, 94, 85, 27 and 34 trials were included to buffer supplementation effects on dry matter intake (DMI), milk yield (MY), fat, crude protein (CP), solid not fat (SNF) and lactose, respectively. The magnitude of the effect (effect size) was assessed using standardized mean differences (SMD) for continuous results, between the buffer supplementation addition and control treatments. The addition of buffer supplementation had no significant effect on DMI (SMD=-0.002, P=0.16), MY (SMD=0.001, P=0.99), CP (SMD=-0.002, P=0.34) and SNF (SMD=0.006, P=0.32), respectively. Milk yield increased in the group receiving the buffer supplementation in comparison with the control group. The percentage of fat (SMD=-0.185, P=0.001) significantly decreased in the control group compared to the group receiving the buffer. The dietary buffers significantly increased the content of lactose (SMD=0.008, P=0.014) in dairy cows' milk. This meta-analysis indicated that buffer supplementation improved milk yield and composition in dairy cows.

**KEY WORDS** buffer supplementation, dairy cows, dry matter intake, milk yield.

## INTRODUCTION

Dairy cows with a high genetic potential for milk yield receiving diets lacking in specific nutrients (e.g., protein, energy, vitamins or minerals) would result in suboptimal production response. To prevent from this happening, producers provide dairy cows with highly digestible diets containing a high proportion of readily fermentable carbohydrates (Plaizier *et al.* 2008). The use of such diets with limited amounts of effective fiber might result in changes in rumen volatile fatty acids (VFA) profiles that may increase rumen acidity (Krause and Oetze, 2006). Buffer supplementation is routinely used to prevent metabolic disorders and reduce

rumen acidity and provide a more favorable environment for microbial activity (Harrison *et al.* 1989). Also, buffers could be preventing an over growth of acid tolerant *Lactobacilli*, preventing the potential reduction in rumen pH (Enemark, 2008). The response to buffer supplementation is variable, it's to be dependent on the inherent buffering capacity of the basal diet. Okeke *et al.* (1983) reported that buffer supplementation modifies the ruminal environment by maintaining pH. Belibasakis and Triantos (1990) showed that the addition of dietary buffers such as sodium bicarbonate (NaHCO<sub>3</sub>), potassium bicarbonate (KHCO<sub>3</sub>), sodium sesquicarbonate, potassium carbonate (K<sub>2</sub>CO<sub>3</sub>) and sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) to high concentrate, restricted

forage diets of cows in early lactation can be useful for milk production. Sodium bicarbonate increased DMI (Nori *et al.* 2010; Shams Al-dain *et al.* 2014; Cruywagen *et al.* 2015), milk production (Sarwar *et al.* 2007; Neiderfer, 2017), and milk fat content (Cabrita *et al.* 2009; Rauch *et al.* 2012) of cows during early lactation when they were fed corn silage as the major source of forage. The results of study by Cabrita *et al.* (2009) showed that the addition of buffers did not affect productive responses in dairy cows, but dietary treatments had only small effect on most milk fatty acids. Rauch *et al.* (2012) showed that sodium bicarbonate buffered the rumen and/or improved acid base balance by increasing dietary cation-anion difference (DCAD). In another study by Razzaghi *et al.* (2020), the milk proportions of trans-10 18:1 and total trans fatty acid dropped significantly with a commercial buffer mix supplementation versus cows fed control treatment. Shire and Beede (2013) suggested that supplementation of cationic salts may improve milk production performance by affecting several biological processes including ruminal buffer capacity and pH, as well as lower the ruminal production of trans fatty acid intermediates. Also, sodium sesquicarbonate, sodium bicarbonate, and a blend of bicarbonate buffers increased C18:2 in milk fat when compared with the control group. The reported values for the effects of buffer supplementation on milk production and composition in dairy cows is different and because the studies investigated on the effect of buffering supplementation were done under different conditions and with different sample size and it has different results, the combining, competition and summary of the results of these studies and achieving a general conclusion to evaluated the real effects of buffer is possible only by a meta-analysis. The objective of this study was to conduct a comprehensive meta-analysis of the effects of buffer supplementation inclusion in the diet of dairy cows, on dry matter intake, milk yield and composition. A subsequent objective was to examine the existence of heterogeneity and publication bias among the studies.

## MATERIALS AND METHODS

### Data collection

The search for information focused on studies with buffer supplementation in different type on dry matter intake, milk yield and composition. The publications were obtained from searches in different databases such as PubMed, Scopus, Web of Science, and Google Scholar. Search strings consisted of words associated with the particular topic, in combination with the use of operators ('and' or 'or'). In the extensive search, the occurrence of all terms within a string was checked by title, abstract, and keywords (i.e., the 'topic' option in the 'Web of Science' and 'ALL' for all

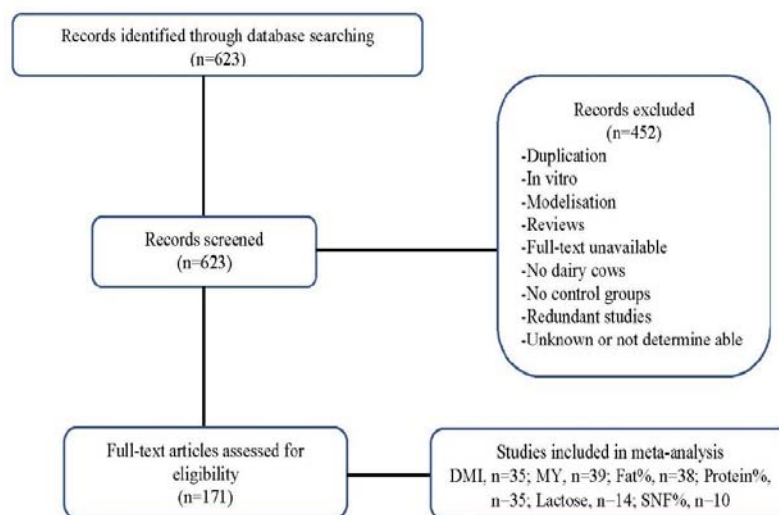
Terms or 'TITLEABS- KEY' for title, abstract and keywords in Scopus). The search was performed using the following terms: "dairy cattle" and "dairy cow", "milk yield", "milk composition" and "performance", "buffer supplementation".

### Inclusion criteria

The inclusion and exclusion criteria followed Lean *et al.* (2014). To be included in the present meta-analysis, the studies needed to be controlled experiments evaluating buffer supplementation in dairy cows and reporting the following information: (1) procedure used to randomly assign animals to treatment and control groups; (2) least square means of the control and experimental groups with variability measures (standard error or standard deviation); and (3) sample size. In total 86, 91, 94, 85, 27 and 34 trials were included to buffers effects on dry matter intake (DMI), milk yield (MY), fat percentage, crude protein (CP) percentage, solid not fat (SNF percentage) and lactose percentage, respectively, between 1969 and 2020, (Figure 1).

### Meta-analytical procedure

The effect size was determined as a standardized mean difference (SMD), by using the methods described by DerSimonian and Laird (1986) for random-effect models. When studies reported variables in the same unit of measure, the raw mean difference was calculated, which allows the interpretation of the effect size in the original units (Appuhamy *et al.* 2013). Forest plot was made to show the impact of buffer supplementation on response variables. In the forest plot, each study is represented by a point with its interval of confidence. Furthermore, the forest plot reports the effect size and weighted contribution to each study from random-effect models. The DerSimonian and Laird (1986) approach to random-effects meta-analysis uses a simple moment-based estimate of the among-study variance (heterogeneity) of the true effects ( $\theta$ ). Heterogeneity was reported using the  $I^2$  statistic. Heterogeneity among studies was assessed using the DerSimonian and the Laird test (Q-statistic). The degree of heterogeneity was quantified with the inconsistency index ( $I^2$ -statistic; Higgins and Thompson, 2002). The presence of publication bias was investigated using funnel plots. An adjusted rank correlation test using the Egger method (Egger *et al.* 1997) and the Begg's test (Begg and Mazumdar, 1994) was used to survey publication bias. Bias was considered to be present if at least one of the statistical methods was significant ( $P < 0.05$ ). If there was any evidence of publication bias, from either the statistical tests or the funnel plot, the "trim-and-fill" method (Duval and Tweedie, 2000) was used to estimate the quantity and magnitude of missing studies and resultant unbiased effect size.



**Figure 1** Study selection flow diagram. DMI: dry matter intake; MY: milk yield; SNF: solid not fat

## RESULTS AND DISCUSSION

The effect of dietary buffers in the diet of dairy cows on DMI, MY, fat, CP, lactose and SNF are presented in Table 1 and Figures 1-7. The use of buffer supplementation had no significant effect on DMI, MY, CP and SNF ( $P>0.05$ ). The DMI decreased (SMD=-0.002;  $P=0.16$ ,  $I^2=70.16$ , Table 1; Figure 2) in the control the group in comparison to group receiving buffer supplementation. Similarly, no significant ( $P>0.05$ ) difference between the control and supplemented dairy cows in relation to the CP content was observed. The addition of buffer had no significant effect on MY (SMD=0.001,  $P=0.99$ ). The content of protein decreased ( $P=0.34$ ) in the control group, with the SMD being -0.002 from a total of 85 trials analyzed. The heterogeneity for CP content was highest ( $I^2=74.10$ ). Positive effects of buffer supplementation were also observed in lactose and SNF contents, with an effect size of +0.008 and +0.006 for lactose and SNF content, respectively. The results had a high level of heterogeneity ( $I^2>50%$ ). No publication bias was evident ( $P>0.05$ ) for these response variables (milk yield, fat, CP and SNF, Table 1). Also, there were publication bias for variables DMI ( $P=0.002$ ) and lactose content ( $P=0.04$ , Table 1).

In early lactation, high producing dairy cows frequently fail to consume adequate feed and generally are in negative energy balance. Increasing the proportion of concentrates above 60% of total ration DM to give higher energy density might bring about numerous problems, for example, rumen acidosis, reduction in forage digestibility, milk fat depression, and possible increase in the incidences of abomasal displacement, milk fever, and ketosis (Clark and Dvise 1980). There was no significant different between treatments on DMI, milk yield, CP and SNF percentage.

Similarly, Cabrita *et al.* (2009) were no observed significant difference in DMI. In contrast to our finding, Thomas and Emery (1969), reported that concentrate consumption decreased, when  $\text{NaHCO}_3$  and  $\text{MgO}$  was added to concentrate portion of the diet. Besides, a lack of response to the added buffer supplementation can be, due to the fairly high fiber content of the total ration. Increased DM in dairy cows fed buffer supplementation might be attributed to higher rumen pH (West *et al.* 1987; Tucker *et al.* 1988), blood  $\text{HCO}_3$  (Shahzad *et al.* 2007), and acid base balance (Sanchez and Beede, 1994).

Okeke *et al.* (1983) reported that buffer can be modify the ruminal environmental by maintaining pH within an optimal range and by increasing the dilution rate of the ruminal fluid. In addition, Hu *et al.* (2007) reported that use of buffers such as  $\text{NaHCO}_3$  increased DCAD and that DCAD and production by dairy cows are related. On the other hand, the DCAD of the diet affects acid-base balance regulation, which in turn affects DMI of dairy cows when  $\text{NaHCO}_3$  is added to the diet. Similar findings were reported by Rogers *et al.* (1982) who observed increased DMI in dairy cows when high sodium bicarbonate was supplemented.

They expressed that as well as buffering effect, sodium bicarbonate also increased ruminal osmotic pressure and liquid dilution rate. Erdman (1988) reported positive responses in dry matter intake and productively for cows' feeds that are low in effective fiber. In rumen sodium bicarbonate is converted into sodium (Na) and bicarbonate ( $\text{HCO}_3$ ) and they impart non-buffering and buffering effects, respectively (Schneider *et al.* 1986). The effect of buffers on DMI depends on the nature of dietary ingredients, their buffering capacity, and the ratio of forage to concentrate (Erdman, 1988).

**Table 1** Effect of dietary buffer supplementation on dry matter intake (DMI), milk yield, and milk composition in dairy cows

Item	Trials	Year	Effect size (95% CI)	P-value	Heterogeneity <sup>1</sup>			Funnel test <sup>2</sup>
					I <sup>2</sup>	Q-value	P-value	
DMI	86	1980-2020	-0.002 (-0.004-0.001)	0.16	70.16	284.9	0.001	0.002
Milk yield	91	1969-2020	+0.000 (-0.09-0.91)	0.99	54.66	198.51	0.001	0.497
<b>Milk composition (%)</b>								
Fat	94	1965-2020	-0.185 (-0.25-0.12)	0	89.90	10133	0.001	0.223
Crude protein	85	1965-2020	-0.002 (-0.006-0.002)	0.34	74.1	324.39	0.001	0.333
Lactose	34	1990-2020	+0.008 (0.002-0.014)	0.014	64.6	93.22	0.001	0.045
SNF	27	1965-2020	+0.006 (-0.006-0.017)	0.32	78.8	122.64	0.002	0.28

<sup>1</sup>I<sup>2</sup> (I-squared) is measure of heterogeneity of random model (RM). Effect size is the estimated standardized mean difference of the mixed model. P-value is the statistical significance of the random model.

<sup>2</sup>Egger's regression asymmetry test.

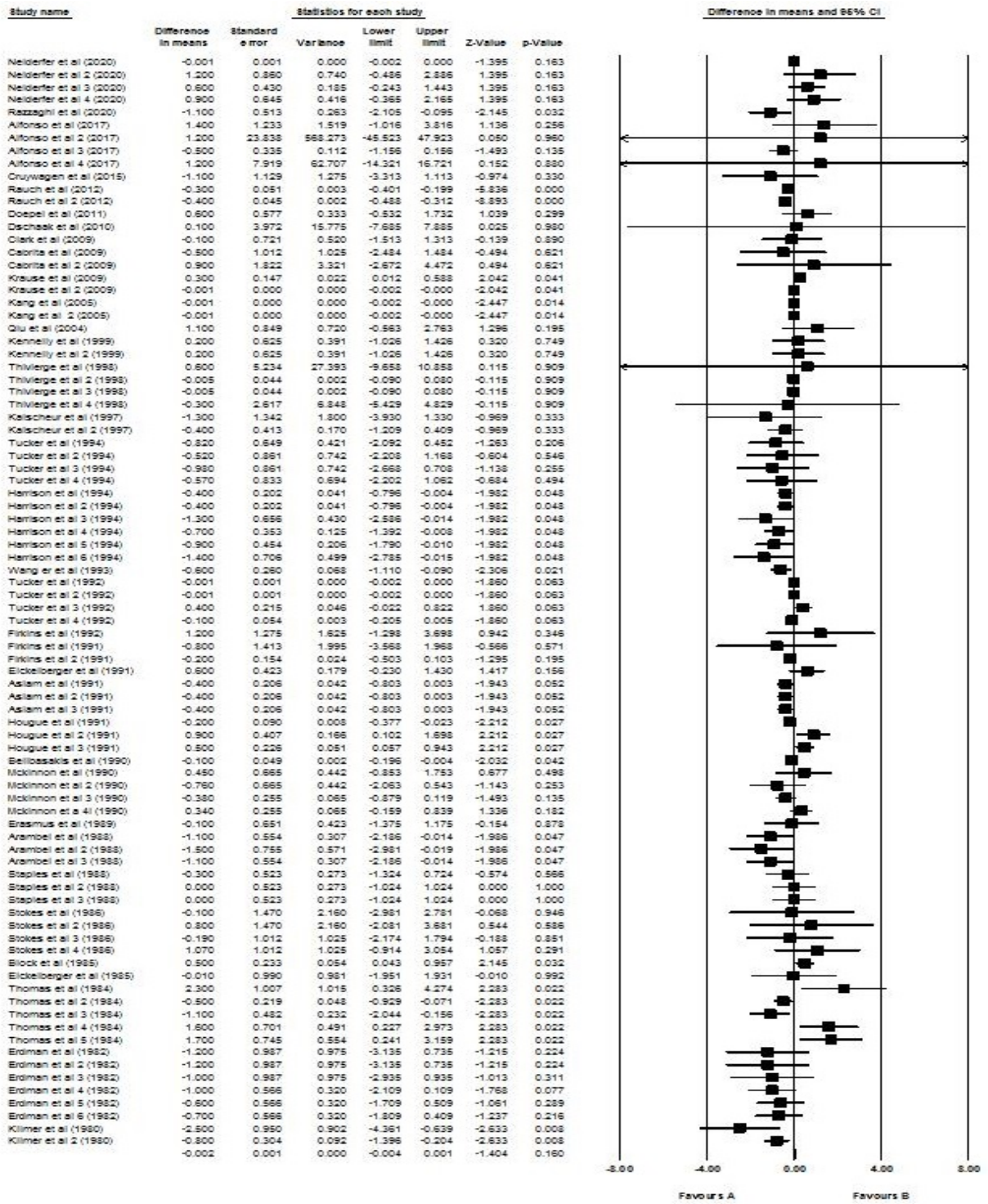
DMI: dry matter intake; SNF: solid not fat and CI: confidence interval.

The lack of effect on milk yield presumably reflects the lack of differences in DMI, but numerically use of buffer supplementation increased milk yield in group receiving buffer. On the other hand, if buffer supplementation altered DCAD and improve acid-base balance, which increased DMI, this can be explain a part of the increase in the production of milk (Clark *et al.* 2009). Our results agree with the finding of Cruywagen *et al.* (2015). These results are supported by Tucker *et al.* (1988) who reported increased milk production in lactating cows fed high sodium bicarbonate compared to those fed a low sodium bicarbonate diet. Our data is an agreement with the finding of Block (1994) who showed that high Na or K contents from sodium or potassium bicarbonate increased milk production in lactating cows. Bougouin *et al.* (2018) did not observe any change in milk yield in cows kept on high starch and high roughage-based rations with supplementation of 1% sodium bicarbonate. He further expressed that lactating cows had a higher metabolic rate that would in general in make the cellular environment acidic due to more CO<sub>2</sub> production. Responses to buffers with other diets are less consistent and appear to depend on factors such as buffer source, the acidity and buffering capacity of diets, and the metabolic acid load of the cow (Meschy *et al.* 2004; Hu and Murphy, 2005). In the present study, milk fat percentage was affected by treatment and was lower in control group. Changes in milk percentage was likely due to an increase in DCAD and/or a rumen buffering effect. Also, Low-fat milk is a result of diets with a high proportion of readily digestible carbohydrates to the fibrous component that can create unfavorable conditions within the rumen (Alfonso-Avila *et al.* 2017). Additionally, when evaluating effects of buffers on alleviating milk fat depression, some results suggest an effect of buffers on rumen biohydrogenation of fatty acids, primarily reducing production of trans octadecenoates in

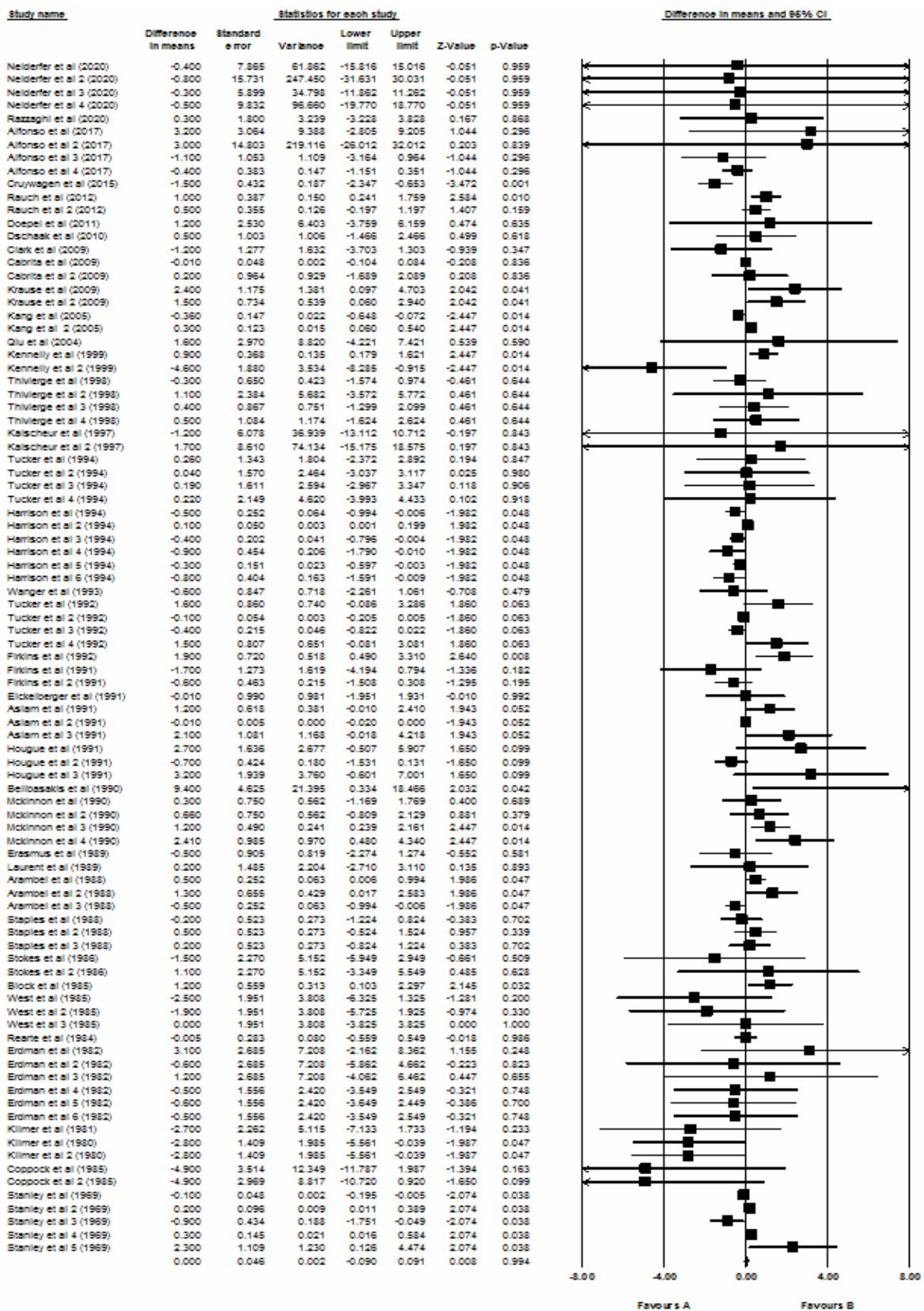
the rumen (Kalscheur *et al.* 1997). On the other hand, the primary reasons buffers are fed are to alleviate milk fat depression and encourage feed intake. High concentrate rations favor a rumen environment that supports propionate rather than acetate production. Grummer (1991) reported that the fat percentage of milk influenced by several factors, including breed, lactation stage and composition of feed of rations.

Also, the reduced milk fat content is frequently used in farms as an indicator of Sub-acute ruminal acidosis (SARA) (Mertens, 1997; De Brabander *et al.* 2002). Erdman (1988), many studies have shown an increase in milk fat when cows are supplemented with buffers. Likewise, it has been shown that the acetate to propionate ratio (A:P) can be increased through buffer supplementation. Newbold *et al.* (1989) reported that the supplementation of NaHCO<sub>3</sub> to diets containing grass silage and molasses did not effect the DMI of primiparous cows in early lactation. The study results Clark *et al.* (2009) showed that use of sodium sesquicarbonate in diet did not affect DMI or DMI as a percentage of BW or BW and body condition score (BCS) during the complete 308-d lactation experiment.

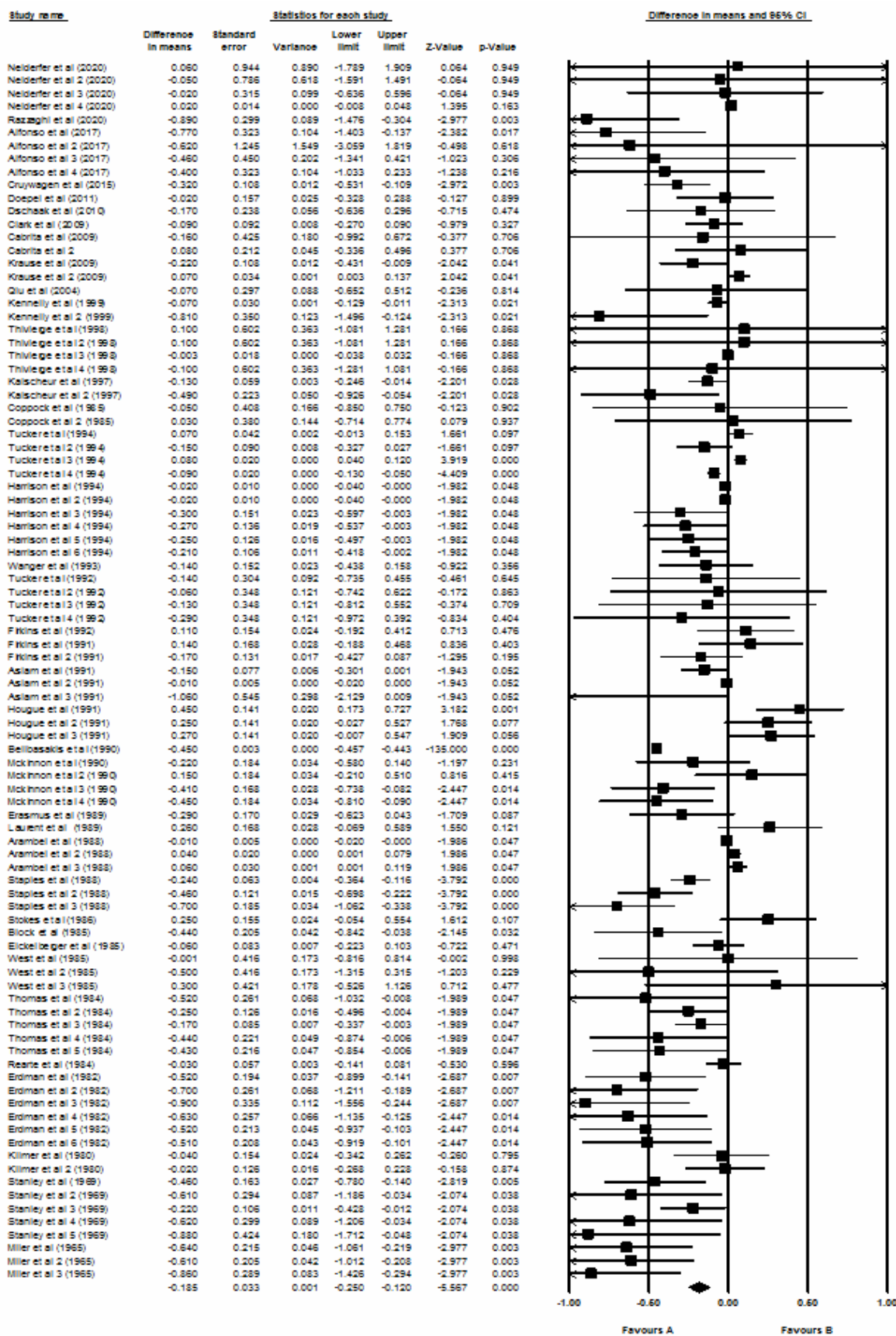
In another study, Cruywagen *et al.* (2015) reported that use of sodium bicarbonate (0.8% of dietary DM) increased DM intake in the comparison control group. Sarwar *et al.* (2007), assessment of the varying level of sodium bicarbonate on milk yield and its composition in Buffaloes showed total milk solids were higher in Buffaloes fed sodium bicarbonate in compared control group. Lactose and SNF percentages were higher for cows receiving buffered diets compared with the control diet. When cows received high concentrate diets (75% concentrate), Khorasani and Kennelly (2001) reported milk fat content to be 4.09% when diets were buffered compared with 2.91% when diets were not buffered.



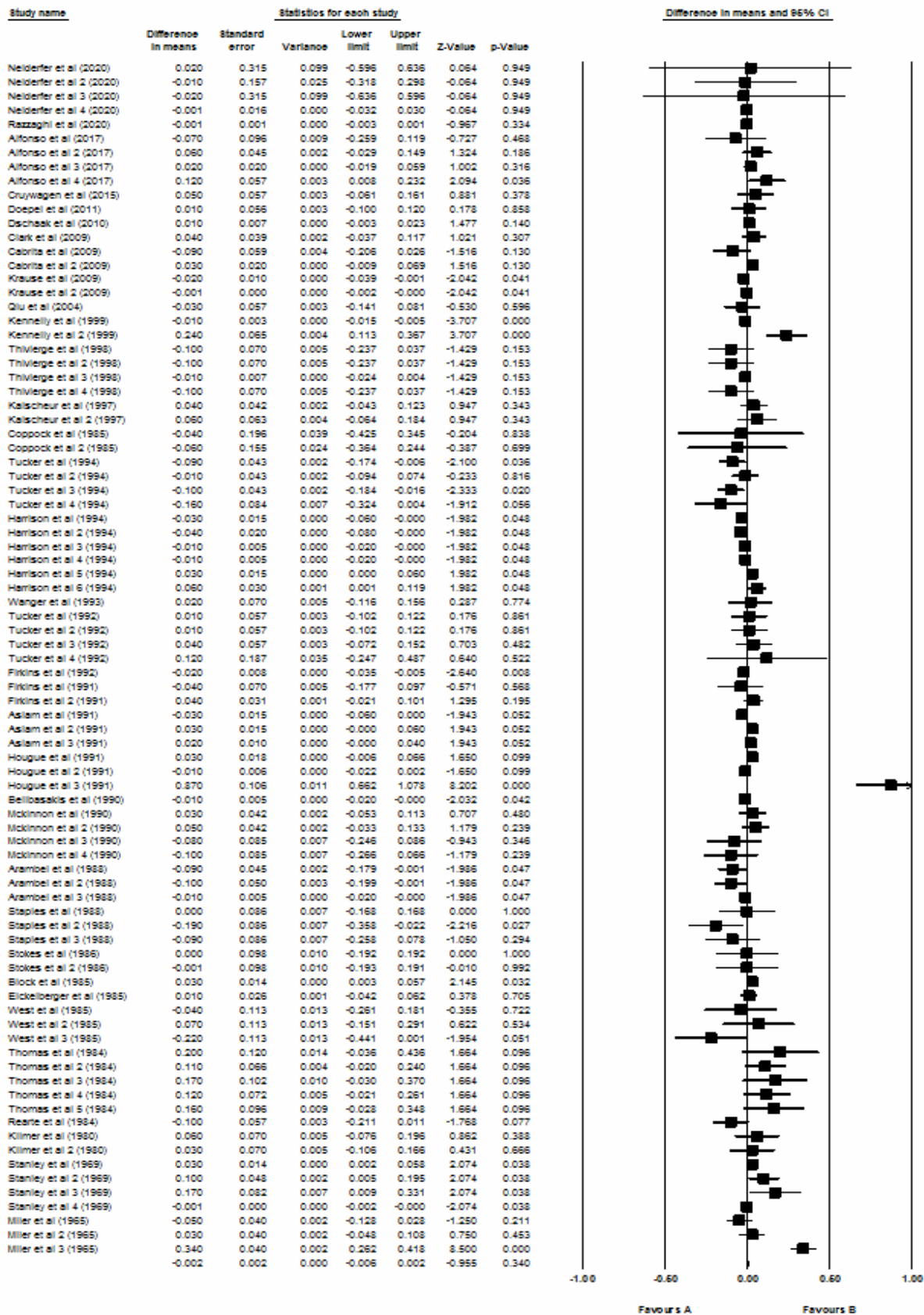
**Figure 2** Forest plot of effect size or standardized mean difference and 95% confidence interval of buffer-supplemented on DMI in dairy cows. B: buffer-supplemented and A: no supplemented. The size of the squares illustrated the weight of each study relative to the mean effect size, which is indicated by the diamond at the bottom



**Figure 3** Forest plot of effect size or standardized mean difference and 95% confidence interval of buffer-supplemented on MY in dairy cows. B: buffer-supplemented and A: no supplemented. The size of the squares illustrated the weight of each study relative to the mean effect size, which is indicated by the diamond at the bottom

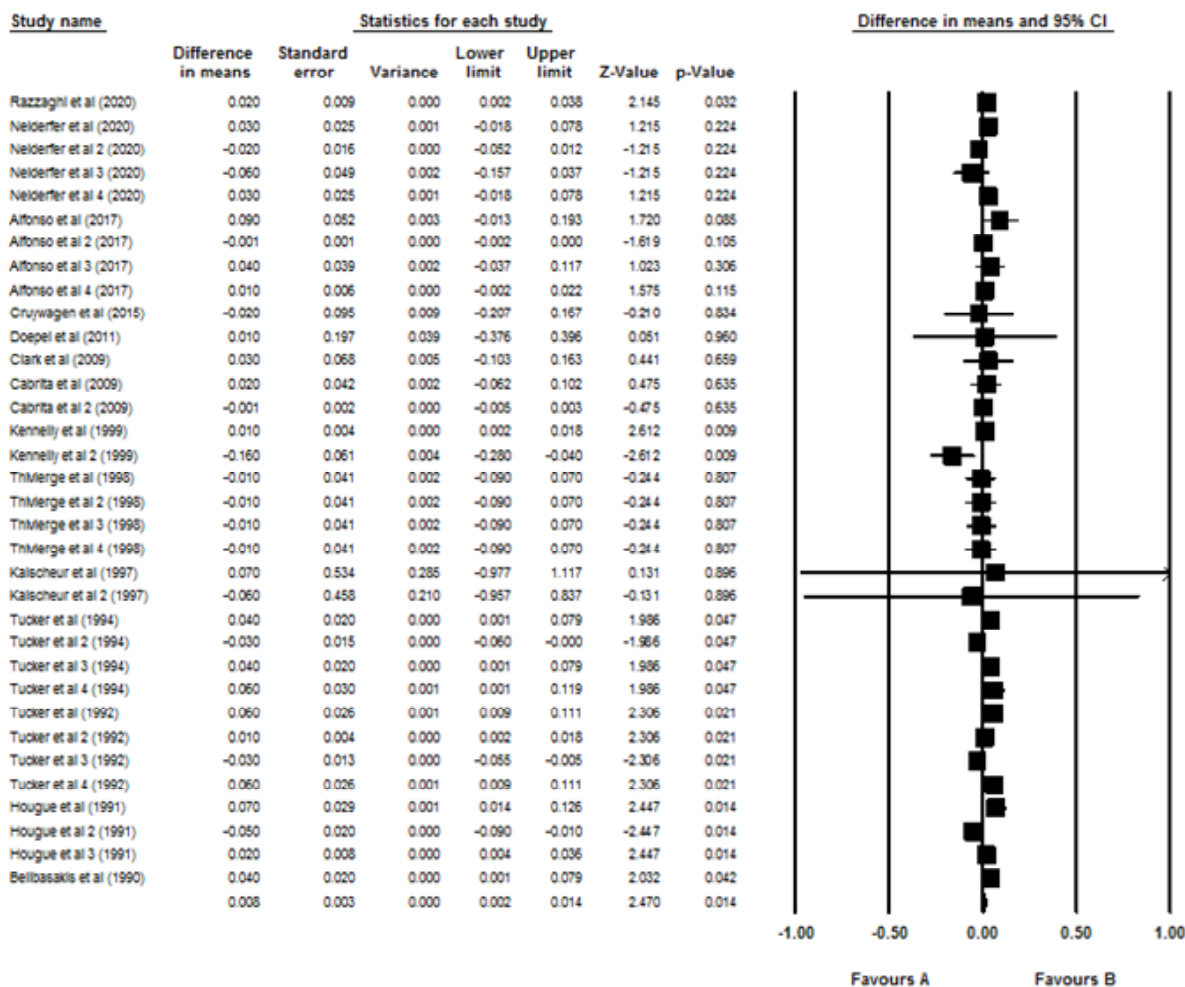


**Figure 4** Forest plot of effect size or standardized mean difference and 95% confidence interval of buffer-supplemented on milk fat percentage in dairy cows. B: buffer-supplemented and A: no supplemented. The size of the squares illustrated the weight of each study relative to the mean effect size, which is indicated by the diamond at the bottom



**Figure 5** Forest plot of effect size or standardized mean difference and 95% confidence interval of buffer-supplemented on crude protein percentage in dairy cows. B: buffer-supplemented and A: no supplemented. The size of the squares illustrated the weight of each study relative to the mean effect size, which is indicated by the diamond at the bottom





**Figure 6** Forest plot of effect size or standardized mean difference and 95% confidence interval of buffer-supplemented on lactose percentage in dairy cows. B: buffer-supplemented and A: no supplemented. The size of the squares illustrated the weight of each study relative to the mean effect size, which is indicated by the diamond at the bottom

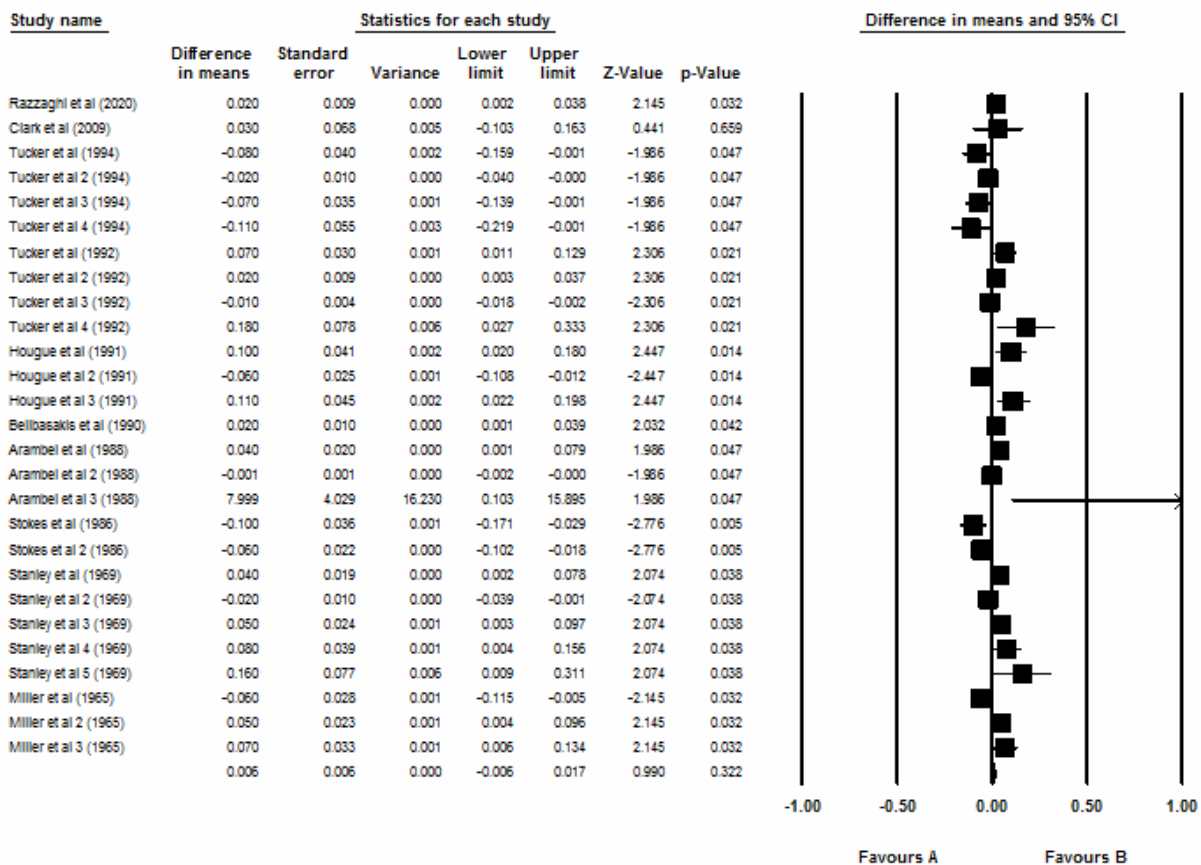
In study [Ghorbani et al. \(1989\)](#), there were no differences among treatments in milk production, milk protein, or 3.5% fat-corrected milk (FCM), however sodium sesquicarbonate increased milk fat percentage (3.89, 3.94, 4.06%) compared with that of the control. Milk protein percentage and solid not fat was not significantly affected by treatment. Diet can influence the production of milk protein more than it can influence milk production content ([Schingoethe, 1996](#)). Protein is one of the most important elements determining milk prices. A diet containing high levels of readily fermentable carbohydrates may increase milk protein content by 1 to 2 g/kg, and could increase the production of milk and protein, but may also result in digestive and metabolic upsets ([Schingoethe, 1996](#)). Improvement in diet composition offer the quickest and sometimes the largest potential for lifting milk protein percentage.

[Thomas and Emery \(1969\)](#) showed that use of sodium bicarbonate and magnesium oxide did not affect milk protein content. But in the current experiment, the numerically use of buffer supplementation improved milk protein percentage in compared control group.

[Zali et al. \(2019\)](#) indicated that the use of a high buffering capacity buffer containing k can increase milk protein percentage in the group receiving buffer supplementation in the compared control group.

[Erdman \(1988\)](#) stated that dietary buffer requirements should change depending on forage particle size, forage intake and ration acidity.

The results revealed SNF percentage of the milk had a significant decrease in the control group, and the results of this experiment are supported by [Alihag Musa and Pandey \(2017\)](#).



**Figure 7** Forest plot of effect size or standardized mean difference and 95% confidence interval of buffer-supplemented on SNF percentage in dairy cows. B: buffer-supplemented and A: no supplemented. The size of the squares illustrated the weight of each study relative to the mean effect size, which is indicated by the diamond at the bottom

Yousef *et al.* (1969) reported that increasing the energy nutrition of the cow increases the SNF content of milk, largely by more milk protein. Zali *et al.* (2019) observed an increasingly significant SNF content in the group receiving a high buffering capacity buffer containing K (HBK). The results show significant increasing lactose percentage in cow’s milk fed buffer supplementation.

The result of this study is supported by Alihag Musa and Pandey (2017) and Rauch *et al.* (2012). Among milk compositions, lactose is the main carbohydrate in mammals’ milk, and it is responsible for the mammary gland (Costa *et al.* 2019). Also, lactose is a disaccharide sugar that is made up of glucose and galactose molecules (Costa *et al.* 2019). It is the major bovine milk solid, and its synthesis and concentration in milk are affected mainly by udder health and the cow’s energy balance and metabolism (Pollott, 2004; Costa *et al.* 2019).

### CONCLUSION

The differences between studies are removed by meta-analysis, which can make the corrected data comparable, creating more objective and convincing data. Although there is inconsistency report from different authors concerning feeding of buffer supplementation, but the overall results of this study revealed that milk fat and lactose percentage significantly influenced by buffer supplementation. But buffer supplementation has no significant difference on DMI, MY, CP and SNF percentage.

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