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Exploring the benefits of β-mannanase supplementation in dairy cattle nutrition, performance, and a sustainable environment

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ABSTRACT

The use of enzymes to improve nutrient utilisation in ruminants has gained significant attention due to their catalytic effects on forages, in breaking down complex polysaccharides such as β-mannan. Cattle typically exhibit low feed efficiency due to the indigestible cell wall contents in their diets, making the inclusion of enzymes like β-mannanase essential. This review explores the benefits of β-mannanase supplementation on the performance, health, and environmental sustainability of dairy cattle. β-mannanase enhances nutrient utilisation by hydrolysing β-mannan, which improves feed efficiency, milk yield, and gut health. By reducing the viscosity of digesta and lowering the energy required for digestion, β-mannanase facilitates more efficient nutrient absorption, leading to enhanced animal productivity. Studies have demonstrated that β-mannanase supplementation can increase milk production by up to 30 - 35% in dairy cows, although it may occasionally result in milk fat reduction. Additionally, β-mannanase has been linked to improved udder health, as evidenced by reduced somatic cell counts in milk, which is indicative of lower mastitis risk and overall animal health. Beyond its benefits to cattle, it contributes to environmental sustainability by reducing nitrogen and phosphorus excretion, thereby mitigating the environmental impact of dairy farming. However, the efficacy of β-mannanase is influenced by dosage, dietary composition, and animal breed, with optimal results observed at a 0.1% inclusion rate. Despite its benefits, the application of β-mannanase comes with some challenges. The variability across different studies highlights the need for further research to establish dosages, evaluate long-term safety, and explore species-specific formulations.

Keywords: additive, enzyme, feed efficiency, properties, milk yield

INTRODUCTION

β-mannanase is an exogenous enzyme that hydrolyses structural hemicellulose in forages fed to ruminants. It gained recognition for its enzymatic hydrolysis on β-mannan, which is a complex polysaccharide, aiding in ruminal nutrient utilisation (Long et al., 2022).

β-mannanase is widely used in non-ruminant animals, making it an area of research for further exploration in ruminants. Additionally, the rumen secretes little exogenous enzymes for digestion purposes, allowing ruminants to experience slow digestion (Boraston et al., 2004). β-mannanase improves growth and digestibility in young ruminants such as calves (Jeong et al., 2021). This indicates β-mannanase can improve digestion and nutrient utilisation in mature cattle.

Ruminants can convert low-quality plant materials to usable protein to meet the increasing requirement for protein. However, in animals such as dairy cattle, the presence of cell walls can reduce how well they digest these materials (Hatfield et al., 1999; Jung et al., 2012).

For instance, forage fills the rumen swiftly due to the high amount of neutral detergent fiber (NDF) (NASEM, 2016), and tends to lower the dry matter intake, feed utilisation, and milk production (Phuong et al., 2013). Forages contain compounds like mannan within their cell walls, which display anti-nutritional characteristics in diets due to their insoluble nature (Chauhan et al., 2012). Additionally, cellulose and hemicellulose are the most common polysaccharides, with the latter comprising mannan, xylan, galactans, and arabinans in their increasing proportions, respectively (Lee, 2018). Nonetheless, their importance in the rumen cannot be underestimated since they reduce rumen disorders (Van Soest et al., 1991). Therefore, the utilisation of exogenous enzymes can be a potential way to reduce indigestion, enhance forage utilisation, and improve the financial condition of livestock farmers (Beauchemin and Holtshausen, 2010). β-mannanase breaks mannan to release trapped nutrients (Bangoria et al., 2021), leading to the increased area in the small intestine with a subsequent decrease in digesta viscosity, especially in birds (Mehri et al., 2010).

Studies have shown benefits from the use of feeding β-mannanase in dairy cattle. Benefits range from increased feed conversion efficiency (FCE) (Seo et al., 2016; Tewoldebrhan et al., 2017; Kebreab et al., 2022) to milk yield (López-Ordaz et al., 2020), anti-inflammatory effects (Roque et al., 2019), sustainable environment (Kebreab et al., 2022), and growth rate (Seo et al., 2016). Additionally, β-mannanase supplementation in dairy feeding reduced somatic cell count in milk (Tewoldebrhan et al., 2017; López-Ordaz et al., 2020), which indicates economic benefits for dairy farmers. However, no effect was found for growth and health in calves supplemented with β-mannanase (Jeong et al., 2021) and lactating cows (Cordero-Vargas et al., 2023). Currently, limited data reports the supplementation of β-mannanase in dairy cattle; however, the literature available displays beneficial results. The objective of this paper is to critically review the effects of β-mannanase application in dairy cattle, its properties, and benefits in dairy farming. Additionally, it examines how supplementing β-mannanase in dairy cattle contributes to a sustainable environment.

SOURCE AND PRODUCTION OF β-MANNANASE

Mannanases are fibrolytic enzymes that act on mannans made up of D-mannose units (Chauhan et al., 2012; Bangoria et al., 2021). They are abundant in nature and are produced from varieties of bacteria, fungi, actinomycetes, and yeasts (Puchart et al., 2004; Dawood and Ma, 2020). This characteristic enables their utilisation for different purposes (Mohapatra, 2021). The major producers of mannanase are microbes, and they remain the desired sources of enzymes due to their characteristic fast growth, less cultivation space and genetic manipulation (Dhawan and Kaur, 2007; Chauhan et al., 2012). Table 1 shows mannanase-producing microbes. In bacteria, the *Bacillus* species are mainly used to produce mannanase, making them the most mannan-degrading bacteria (Singh et al., 2010; Regmi et al., 2016; David et al., 2018). Although it has been reported that *Klebsiella oxytoca*, a gram-negative bacterium, also engages in mannanase production (Titapoka et al., 2008; Pongsapipatana et al., 2016). Among the fungi, the genus *Aspergillus* is well-known for mannanase production (Norita et al., 2010; Adesina et al., 2013; Liu et al., 2020). Also, actinomycetes, specifically *Streptomyces* species, are known to have produced mannanase (Bhoria et al., 2009; Pradeep et al., 2016).

Microbial production of mannanase is done outside of the cells using different substrates (Dhawan and Kaur, 2007). The most common substrate for inducing β-mannanase is the locust bean gum (Xie et al., 2020). Other materials like konjac powder, copra meal and wheat bran have also been used for the induction of mannanase because of their readily availability and affordability (Singh et al., 2010; Bangoria et al., 2023a). Similarly, Luo et al. (2018) suggested that non-pretreated crude glycerol with yeast (*Pichia pastoris*) might be used for producing β-mannanase. In addition, Chauhan et al. (2012) reported that microbial production can be done through cloning of bacterial or fungal genes. In their case, production is for commercial purposes and as such is more uneconomical compared to simple and less expensive medium ones (Luo et al. 2009).

Table 1. Source of β-mannanase

Organism
Klebsiella pneumoniae SS11
Bacillus subtilis HM7
Lactobacillus casei HDS-01
Streptomyces spp. Alg-S25
Penicillium aculeatum APS1
Rhizopus microspores
Aspergillus sulphureus
Lichtheimia ramose
Trichoderma longibrachiatum RS1
Bacillus sp. R2AL2A
Aspergillus niger CBS 513.88
Aspergillus terrus
Aspergillus kawachii IFO 4308
Paenibacillus polymyra
Paenibacillus curdlanolyticus

Adapted from "Industrial and Biotechnological Use of β-mannanase Enzyme" by Georgiev (2023)

Based on related characteristics of amino acids, Henrissat (1991) identified two microbial families encoding β-mannanase. They are metabolic protein ManA from *Caldicellulosiruptor saccharolyticus* (Lüthi et al., 1991) and ManA from a *Bacillus* species (Akino et al., 1989). The success of genetic manipulation of microorganisms has enabled the production of desirable mannanases. For example, companies such as P and G, ChemGen and Genecor developed recombinant mannanases for commercial purposes (Dhawan and Kaur, 2007). This shows microbes are heavily used for mannanase production.

PROPERTIES OF β-MANNANASE AND MODE OF ACTION

β-mannanases are enzymes responsible for the breakdown of structural bonds in mannan, a polysaccharide. β-mannanase breaks down mannan oligosaccharides (MOSs) to about 3 to 7 degrees of polymerisation (DP) (Li et al., 2024). Its catalytic efficiency increases with increasing DP (Pongsapipatana et al., 2021). β-mannanase, especially those extracted from fungi and bacteria, need several sites for hydrolysis of mannan (Halstead et al., 1999), releasing mannose and mannotetraiose (Torto et al., 1996). Similarly, studies reported that mannanase released mannobiose (M2) and mannotriose (M3) as the major end products (Civas et al., 1984; Sachslehner and Haltrich, 1999). Unlike the hydrolysis of mannan, they form complex polysaccharide structures via glycosylation by accepting glycosylic bonds (Gübitz et al., 1996; Harjunpää et al., 1999). This was seen in *T. ressei* β-mannanases, forming trans-glycosylation with mannose or mannobiose (Harjunpää et al., 1999). Moreover, β-mannanase operates at different temperatures and pH levels. For example, β-mannanase (IDSGH5-14 (CD) extracted from the rumen of sheep displayed an optimal temperature of 50 °C (Li et al., 2024), whereas some β-mannanases have a higher optimal temperature of 65 °C (as seen in Taman5 mannanase) (Zheng et al., 2023), or 90 °C (Parker et al., 2001). This is due to the inactivation of enzymes above thermal temperature, and the difference in activation energy to denature enzymes (Thomas and Scope, 1998). Li et al. (2024) suggested that the optimal temperature for most β-mannanases obtained from ruminant animals is within the range of 30 to 50 °C, enabling them to have mesophilic and acidic adaptation. Also, β-mannanase functions at a pH value between 3.5 and 9 (Hägglund, 2002; Pongsapipatana et al., 2021). Although most of its activities occur between a pH value of 4 to 7 (Pongsapipatana et al., 2021; Bangoria et al., 2023a; Li et al., 2024). This indicates that a highly acidic level may affect its hydrolytic activity on the substrate, mannan.

Furthermore, β-mannanase has the potential to either accelerate or decelerate hydrolysis processes when combined with metal minerals or chemicals. This depends on the level of concentration of minerals in the enzyme (Zheng et al., 2023). For instance, Fe^{2+}, Fe^{3+}, K^{+}, and Ca^{2+} significantly improved the activity of β-mannanase at

1 mM concentration (Bangoria et al., 2023b; Zheng et al., 2023). Increasing the concentration to 5 mM, Fe^{2+} and Fe^{3+} subsequently improved β-mannanase activity (Zheng et al., 2023). However, a further increase in the concentration (10 mM) resulted in a decrease in its activity by some metal minerals such as Fe^{3+}, Mn^{2+}, and Ca^{2+}, respectively (Zheng et al., 2023). Conversely, K^{+} and Zn^{2+} improved β-mannanase activity at a 10 mM concentration (Bangoria et al., 2023b; Zheng et al., 2023). This suggests that minerals can positively or negatively influence β-mannanase activity, depending on the specific mineral and its concentration.

Mode of action

Generally, β-mannanase randomly acts on linear substrate to release mannobiose (M2) and mannotriose (M3) (Pongsapipatana et al., 2021; Li et al., 2024). It prefers linear substrates to branched substrates (Pongsapipatana et al., 2021), mainly mannan polysaccharides such as galactomannan and glucomannan (Bangoria et al., 2023b; Li et al., 2024). This preference was seen when catalytic residues were placed in different subsites -1 +1 and +2, respectively. M3 structure accesses the above binding sites without delay and forms a hydrogen bond between the 1-subsite mannose residue and amino acid (His114), stabilising the binding. Conversely, a galactosyl-branched structure was prevented by loop 6 (formed around sites via hydrogen bonds for binding purposes) from accessing the sites, showing β-mannanase preference for linear substrate (Pongsapipatana et al., 2021). Also, β-mannanase breaks down the enclosing matrix close to the protein vacuole and cell walls. This allows protein accessible to endogenous enzymes, which could enable optimal utilisation of feed in animals (Nabte-Solis, 2008; Rueckel et al., 2024). The hydrolytic effect on protein could be a result of protease resistance (Bangoria et al., 2023b), indicating mannanase could be useful as a feed additive.

IMPACT OF β-MANNANASE SUPPLEMENTATION ON MILK YIELD AND COMPOSITION

López-Ordaz et al. (2020) investigated the effect of β-mannanase addition on dry matter intake in Holstein-Friesian cows. The results showed that the milk yield of the cows increased, with no effect on dry matter intake among the treatments. This indicates the importance of β-mannanase in dairy farming as a supplement to increasing milk volume. Tewoldebrhan et al. (2017) reported increased milk yield at 0.1% inclusion of β-mannanase in the diet of cows. Recently, Kebreab et al. (2022) reported a higher milk yield (P = 0.05) per yield to DMI ratio. Consequently, the enzyme has positively influenced milk yield. Moreover, Sharma et al. (2021) reported an enhancement in milk composition after being combined with other fibrolytic enzymes. This tends to show the dual purpose of the enzyme by comminution and improving the nutritional constituents of the fed diets. However, Tewoldebrhan et al. (2017) doubled the initial dosage of 0.1% and found that β-mannanase was not effective. Similarly, Cordero-Vargas et al. (2023) reported no effect (P > 0.05) of β-mannanase at 0.1% and 0.2% added to the commercial diet of Jersey cows, respectively. This could mean high concentration or breed may be responsible for β-mannanase effects on milk yield and composition. More studies with higher percentages of inclusion are encouraged.

IMPACT OF β-MANNANASE SUPPLEMENTATION ON THE HEALTH OF DAIRY CATTLE

β-mannanase improves the health of animals mostly via indirect mechanisms. The addition of β-mannanase in the diet breaks down mannan to supply mannose oligosaccharide (MOS), which is beneficial for animal health (Jeong et al., 2021). For instance, it increased to neutrophil-to-lymphocyte ratio in supplemented calves, which could boost the immune system. However, the opposite occurs when combined with another treatment diet like bacteriophage (Jeong et al., 2021). β-mannanase acts as prophylactic, helping to prevent mastitis in lactating cows (Tewoldebrhan et al., 2017), possibly via cells such as macrophages, lymphocytes, and neutrophils

in the somatic cell count (Yu et al., 2011). Although a high somatic cell count (SCC: between 74,000 cells/mL to 120,000 cells/mL) indicates infection in the udder (Nyman et al., 2016). Studies by Tewoldebrhan et al. (2017) and Kebreab et al. (2022) reported SCC of 59 cells/mL in supplemented diets, indicating β-mannanase may improve udder health in cows. Therefore, nutritionists should find a way to include it as a component of the cows' diet.

IMPACT OF β-MANNANASE SUPPLEMENTATION ON DIGESTIBILITY IN DAIRY CATTLE

Despite some positive responses from using β-mannanase as a supplement to dairy cattle, few or no significant effects have been observed on digestibility. Previously, improved FCE has been reported, which is correlated to digestibility (Tewoldebrhan et al., 2017). However, a recent study observed no difference in digestibility among treatment groups, although digestibility for organic matter (OM) and ash tended to be higher in supplemented cows at 0.1% (Kebreab et al., 2022). However, research carried out by Tewoldebrhan et al. (2017) indicated that digestibility (dry matter: DM, acid detergent fiber: ADF, neutral detergent fiber: NDF, crude protein: CP, OM, and ash) significantly ($P < 0.05$) decreased in cows treated with low β-mannanase concentration, leading to low absorption of OM and CP (Tewoldebrhan et al., 2017). Cows fed with high β-mannanase doses had digestibility similar to the control, indicating that increasing the percentage of β-mannanase in the diet could improve digestibility in ruminants. However, this should depend on the target variable. Moreover, Lee et al (2014) noticed digestibility was not different in goats fed with treatments, although NDF digestibility tended ($P > 0.05$) to be higher in treated goats. This shows β-mannanase is effective for releasing energy for the rumen microbes by breaking down NDF in fiber diets.

FEED CONVERSION EFFICIENCY AND ENVIRONMENTAL IMPACT

Feed conversion efficiency in a cow shows how nutrients are well utilised for greater output. A report by Kebreab et al. (2022) showed that β-mannanase improved FCE in cows fed the enzyme. For example, β-mannanase significantly ($P < 0.05$) improved FCE by 13.4% in cows fed a supplemented diet when compared with those fed on a high crude protein diet. Similarly, comparing the supplemented diet to the low crude diet showed an improvement in FCE by 11.0% ($P < 0.05$) on a DMI basis (Kebreab et al., 2022). Due to the improvements seen in the low crude protein diet, the authors suggested that β-mannanase could be responsible for the significant difference, and not due to the low protein diet. Feed conversion efficiency improved ($P < 0.05$) in goats fed a diet supplemented with β-mannanase levels (Lee et al., 2014). However, the first study on β-mannanase supplementation in cows showed a tendency ($P > 0.05$) to reduce FCE at varying levels (Seo et al., 2016). Furthermore, López-Ordaz et al. (2020) observed improvement ($P < 0.05$) in FCE in supplemented cows during the pre-partum and postpartum period. Moreover, they noticed no difference in nitrogen content in the milk of the control and treated cows. This suggested an improved FCE. Additionally, a study by Roque et al. (2019) showed that mannanase supplementation at 0.1% DM did not affect the FCE of treated cows. This tends to indicate the enzyme could be added to cow diets without concern, bearing in mind FCE may not be reduced. Nonetheless, it will be more beneficial if used in a low-mannan diet (Seo et al., 2016). They suggested that cows fed a diet containing low mannan content tended to have higher average daily gain than those fed a high mannan diet. This suggests high amounts of mannanase in a mannan mannan-supplemented diet may have adverse effects on cattle.

High release of minerals such as nitrogen (N) and phosphorus (P) via the urine of animals contributes negatively to the environment. Feeding cows with β-mannanase supplemented in feed reduces the level of N

and P release into the environment (Kebreab et al., 2022). Nitrogen level in faeces and urine was reduced ($P < 0.05$) in low crude protein (LCP) and mannanase-supplemented feed (Kebreab et al., 2022). The reduced N in LCP might be a result of low protein; however, the N level in the diet supplemented with mannanase was lower, suggesting the effect of β-mannanase. In contrast, Tewoldebrhan et al. (2017) reported a non-significant effect on nitrogen output from cows. The nitrogen concentration increases with supplementation (199 g and 195 g, respectively) and tends to decrease as β-mannanase concentration increases further. Similarly, Cordero-Vargas et al. (2023) also used similar concentrations (0.1% and 0.2%) of β-mannanase utilised by Tewoldebrhan et al. (2017). They observed nitrogen content decreased with increasing β-mannanase concentrations (18.20 mg/dl and 17.30 mg/dl, respectively) compared with the control (18.83 mg/dl). This is an indication that the enzyme could contribute to improving the environment via the nitrogen reduction in urine. Nitrogen content in the milk is also used to predict nitrogen excretion. The study by López-Ordaz et al. (2020) reported that β-mannanase supplementation does not influence the milk nitrogen content, suggesting that more trials evaluating the effects of β-mannanase on nitrogen output need to be conducted.

Also, the phosphorus output was lower in β-mannanase-supplemented diets than in LCP and high crude protein diets (Kebreab et al., 2022). In comparison, Tewoldebrhan et al. (2017) used different concentrations of 0.1% and 0.2%, respectively and observed that P output was higher (79 g) in cows fed 0.1% than in cows (75 g) treated with 0.2%. The reduction of P in cows fed 0.2% β-mannanase was a positive effect compared with the control (77 g). However, the reason for the increased P in those with supplementation was unknown. Also, Cordero-Vargas et al. (2023) reported a non-significant effect for P content. Unlike P data from Tewoldebrhan et al. (2017), the P concentration was slightly higher (162.1 mg/L) in cows fed 0.2% β-mannanase than in cows (159.6 mg/L) that received 0.1% β-mannanase. In contrast, P output was lower in Cordero-Vargas et al. (2023) than in Tewoldebrhan et al. (2017), which is a good indicator that β-mannanse could improve P utilisation. Future research needs to focus on mineral excretion since an improvement in milk yield has been reported.

DOSAGE AND COMPATIBILITY OF β-MANNANASE WITH OTHER ADDITIVES

Different amounts of β-mannanase have been used in the supplementation of dairy diets. Most researchers used a dosage of β-mannanase based on the percentage DM of the feed. For example, 0.1% and 0.2% have been used on DM basis, respectively (Tewoldebrhan et al., 2017; Roque et al., 2019; Jeong et al., 2021; Kebreab et al., 2022). A dosage of 0.1% (the most used dosage) was more effective in dairy cattle compared to 0.2%, indicating the efficacy of the enzyme (Tewoldebrhan et al., 2017; Kebreab et al., 2022). Similarly, a higher dosage, 0.3% was supplemented and compared with 0.1% in feeding young Korean goats. The latter β-mannanase concentration significantly ($P < 0.05$) improved the average daily gain in goats (Lee et al., 2014). Therefore, a 0.1% dose of β-mannanase in relation to DM may be considered an optimal amount during supplementation of cattle diets.

There are few studies on combining β-mannanase and additives in dairy trials including non-ruminant animals. The first combination of β-mannanase with bacteriophage in young calves was reported by Jeong et al. (2021). They recorded that no combined effects ($P > 0.05$) were seen from the combined supplementation of β-mannanase and bacteriophage in calves. However, individually, β-mannanase enhanced intake ($P < 0.01$) with the tendency to increase body weight gain (Jeong et al., 2021). This suggests β-mannanase may be less effective when used together with an additive in cattle feeding. In contrast, positive effects were reported in laying hens when it was supplemented together with another additive. β-mannanase and probiotics significantly ($P < 0.013$) increased laying rate and overall health compared to the control, and improved association by 13.9% (Carvalho et al., 2023). In addition, β-mannanase and probiotics improved the welfare of laying hens when added to their diets (Carvalho et al., 2022). This indicates β-mannanase is more beneficial in laying hens when

combined with probiotics, unlike in dairy cattle, where sole supplementation tends to be more effective. More research in dairy production involving the combination of β-mannanase with other enzymes is warranted to better understand its synergetic association. Moreover, some synergistic effects of β-mannanase and other additives without using animal trials were reported. For instance, Bangoria et al. (2023a) combined β-mannanase and α-galactosidase (both produced from *Penicillium aculeatum*) on the hydrolysis of galactomannan in a simultaneous mode. They observed that the combination of β-mannanase and α-galactosidase released twice as much reducing sugars from galactomannan compared to the total amount released by each enzyme used individually, indicating a synergistic effect with a degree of synergy of 2 (Bangoria et al., 2023a). In addition, a similar result was reported when both β-mannanase and α-galactosidase were supplemented in palm kernel meal (PKM), releasing 21% of reducing sugar (Xie et al., 2020). Also, commercially, β-mannanase and protease were reported to have more removing abilities than individual abilities when applied in a detergent (David et al., 2018). These suggest β-mannanase may function effectively with another enzyme. This area can be explored in dairy cattle to observe the synergetic effects of β-mannanase and other enzymes.

ECONOMIC BENEFITS OF APPLYING β-MANNANASE IN DAIRY FARMING

Supplementing low crude protein diets with β-mannanase tends to reduce the cost of production. Supplementing diets with exogenous enzymes such as mannanase improved FCE in cattle, indicating farmers could spend less money and still get more milk from their cows (Dutta et al., 2023). For instance, Kebreab et al. (2022) suggested that supplementing low-protein diet with 0.1% β-mannanase could help save $1.03 per cow daily compared to using a high crude protein diet. This will enable dairy farmers to increase profit, and perhaps encourage the use of exogenous enzymes in dairy production (Singh et al., 2018).

CONCLUSION AND FUTURE RECOMMENDATION

This review emphasises β-mannanase supplementation in dairy cattle nutrition. β-mannanase improves nutrient utilisation in cattle by breaking down mannan contained in forages. It improves milk yield, milk composition, and growth performance. The supplementation of β-mannanase aids in environmental sustainability by reducing excess nitrogen and phosphorus excretion into the environment. It reduces the level of nitrogen and phosphorus in both the urine and faeces of animals treated with the enzyme. This can reduce excesses of these minerals into the environment, and reduce adverse effects such as nitrogen emissions and soil degradation. Additionally, it improves cow health via a prophylactic effect on mastitis. Supplementation of β-mannanase in cattle diets enhanced feed conversion efficiency and cost savings, particularly when incorporated into low-protein diets, making it more valuable to dairy farmers. However, less synergetic effects of β-mannanase have been reported in ruminant studies, and more research on β-mannanase and other additives needs to be explored. Despite the reported benefits of β-mannanase supplementation in dairy cattle, several studies observed no difference in milk yield and milk composition across varying β-mannanase concentrations. Therefore, future research should also aim to evaluate optimal dosage and long-term effects on cattle health.

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