# Heat moisture treatment-induced changes in feed: A meta-analysis of effects on in vitro rumen fermentation

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**Abstract.** Heat Moisture Treatment (HMT) is a promising approach to improve the efficiency of concentrate feed in ruminants by inducing changes in feed composition and in vitro rumen fermentation. This meta-analysis evaluates the impact of HMT on feed, focusing on fermentation characteristics and methane emissions. Data from the Scopus database were analyzed using a fixed-effect model, suitable for synthesizing studies with comparable conditions. HMT significantly reduces feed starch content ($p<0.001$), altering its nutritional profile. It decreases total volatile fatty acids (VFAs) ($p<0.05$), including acetate, propionate, and butyrate, and shifts the acetate-to-propionate ratio. These changes, alongside reduced ammonia, suggest benefits for rumen health, such as mitigating acidosis risk and improving feed efficiency. However, HMT negatively affects the digestibility of dry matter and organic matter, which may reduce nutrient availability. No significant effects were observed on crude protein digestibility or methane production. While HMT shows potential to enhance feed efficiency and support metabolic health, the trade-offs between reduced digestibility and fermentation benefits highlight the need for further research. These findings provide insights into balancing nutritional and metabolic outcomes when using HMT in ruminant diets.

## 1 Introduction

Feed plays an indispensable role in improving livestock production efficiency, which is directly linked to food security. Enhancing ruminant productivity can be achieved by strategic advancements in feed, particularly by optimizing the physical and chemical properties of feed ingredients. Generally, the largest component of ruminant concentrate feed is composed of grains and pulses, which serve as essential sources of protein and starch. Unfortunately, in the rumen of ruminants, nutrients from these grains and pulses are easily degraded. This can be particularly problematic for high-producing ruminants, where glucose and protein become limiting nutrients. During such times, ruminants must rely heavily on

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their glucose supply, particularly from gluconeogenic precursors such as propionate and gluconeogenic amino acids [1]. Therefore, it would be advantageous if more starch and protein could bypass ruminal degradation and provide glucose and amino acids that are directly absorbed in the small intestine.

Consequently, researchers have explored various methods to modulate the degradability of starch and protein sources, aiming to enhance nutrient absorption in the small intestine and improve feed efficiency. One such approach is the application of heat moisture treatment (HMT) to animal feed. The mechanism behind HMT improves feed quality by enhancing the digestibility of both starch and protein [2], inducing physical and chemical modifications. This process facilitates the formation of a stable matrix, optimizing nutrient absorption. Moist heat treatments like autoclaving are notably more effective than dry heat methods because the presence of moisture allows for a more even distribution of heat. This promotes the consistent formation of a protein-carbohydrate matrix throughout the feed. According to the meta-analysis by Isra et al. [3], HMT significantly increased resistant starch content and improved the prebiotic properties of high-carbohydrate foods. Furthermore, both the type of heat treatment and the specific temperature and time are crucial factors in maximizing the feed's nutritional value and enhancing its overall utilization.

Previous studies have indicated that HMT can effectively alter the chemical composition of feed, thereby improving its digestibility and reducing methane production. However, the results have been inconsistent. For example, Pambudi et al. [4] and Putra et al. [5] reported an increase in total volatile fatty acids (VFA) during in vitro rumen fermentation, while Xin et al. [6] found no reduction in nutrient degradation in seeds subjected to steam pressure for 60 minutes. When methane production was estimated using the Moss et al. [7] method, Xin et al. [6] reported an increase in methane gas production, whereas Putra et al. [5] observed that HMT applied to cassava starch neither increased nor decreased methane production during 48 hours of in vitro rumen incubation. These inconsistent results warrant a quantitative synthesis to determine the true effects of HMT on feed and its impact on rumen fermentation products, digestibility, and chemical composition.

Meta-analysis on feed processing methods, particularly those involving heat treatment in ruminants, have been reported by other researchers. For instance, Ferraretto et al. [8] found that starch digestibility was improved in dairy cows fed diets containing corn grain that had been ensiled or steam processed. Similarly, Rafiee and Darabighane [9] reported that replacing finely ground corn with steam-flaked corn in diets increased milk protein and reduced milk fat. However, there have been no specific meta-analyses focusing on the effects of HMT on in vitro feed characteristics. Therefore, the present study aims to conduct a meta-analysis to evaluate the effects of heat moisture treatment on feed modifications, specifically focusing on its influence on chemical composition and in vitro rumen fermentation characteristics. By consolidating findings from various studies, this research seeks to clarify the potential benefits of HMT as a viable approach to improving feed efficiency and enhancing the sustainability of ruminant production systems.

## 2 Materials and Methods

The database for this study was constructed by systematically identifying relevant articles on the Scopus platform, utilizing the following keywords: TITLE-ABS-KEY (rumen) AND TITLE-ABS-KEY (feed) AND TITLE-ABS-KEY (autoclave) OR TITLE-ABS-KEY (heat moisture treatment). The article selection process followed the rigorous guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [10], ensuring a thorough and transparent approach. During the screening phase, 59 articles were initially identified, resulting in a final database comprising 24 comparisons derived from 6 selected references, as presented in Table 1.

**Table 1.** Studies included as a database for meta-analysis of modified feed with heat moisture treatment

| Referensi | Cycle | Feed | Pressuare and Time | Temperature (°C) | Cooling |
|---|---|---|---|---|---|
| Pambudi et al. [4] | 1 | Soybean | 1 atm, 60 min | 145 | na |
| Putra et al. [5] | 1,2,3 | Cassava | 1 atm, 15 min | 121 | -20 °C, 6 hours |
| Xin et al. [6] | 1 | Brassica carinata seed | 1 atm, 30 min, 60 min, 90 min | 121 | na |
| Feng et al. [11] | 1 | Barley | 1 atm, 60 min | 120 | 20-22 °C |
| Guney [12] | 1 | Barley | na | 105-170 | na |
| Seifdavati & Taghizadeh [13] | 1 | Common vetch, Bitter vetch, Chickling vetch | 1 atm, 20 min | 127 | na |
| Sveinbjornsson et al. [14] | 1 | Peas | 1 atm, 60 min | 145 | na |

To be included in the database, studies had to meet specific criteria. Eligible papers were those published between 2004 and 2024 that investigated the effects of heat moisture treatment on individual feed ingredients, with a primary focus on in vitro fermentation. The analyzed parameters covered key fermentation indicators such as pH, ammonia (NH3) concentration, total volatile fatty acids (VFA), acetate (C2), propionate (C3), butyrate (C4), the acetate-to-propionate ratio (C2), total gas production at 48 hours, and methane production at 48 hours. Additionally, the studies examined changes in the chemical composition of feed ingredients, including starch and crude protein, as well as nutrient degradation parameters such as dry matter, organic matter, and crude protein.

The data analysis employed the Hedges' methodology to calculate the effect size for each study. These individual effect sizes were then pooled using a fixed-effects model to determine the overall effect size, along with corresponding 95% confidence intervals (CI). Additionally, publication bias was assessed through funnel plots. A comprehensive meta-analysis, including cumulative meta-analysis, forest plots, funnel plots, and Egger's test, was conducted using JASP software to ensure robustness and accuracy of the findings.

# 3 Results and Discussion

## 3.1 Chemical composition

The meta-analysis results on the chemical composition of modified feed induced heat moisture treatment, presented in Table 2. Starch content decreased significantly (estimate = -1.497, $p < 0.001$), indicating that heat moisture treatment reduces starch levels in the modified feed. In contrast, crude protein content showed a non-significant increase (estimate = 0.524, $p = 0.183$), suggesting that protein content is not substantially altered by the treatment. The decrease in total starch content due to HMT is probably a result of interactions among polymer chains that disturb the crystalline structure and break apart the double helical arrangement within the amorphous regions. This disturbance is subsequently followed by a reorganization of the affected crystalline regions [15]. Furthermore, gelatinization under pressure may take place, which can lead to the fragmentation of the amylopectin molecular structure or the breakdown of long-chain amylose into shorter chain molecules [16]. This reduction in starch content correlates with an increase in reducing sugars. Zhu et al. [17] suggest that HMT may be associated with heat-induced cross-linkages between amino acids and reducing sugars, similar to what occurs in Maillard reactions.

**Table 2.** Result of meta-analysis on chemical composition of modified feed with heat moisture treatment

| Parameter | NC | Estimate | Lower | Upper | SE | P-value |
|---|---|---|---|---|---|---|
| Starch (%) | 20 | -1.497 | -2.025 | -0.970 | 0.269 | <0.001 |
| Crude protein (%) | 7 | 0.524 | -0.247 | 1.296 | 0.394 | 0.183 |

NC= number of comparison; SE= standard error.

## 3.2 In vitro rumen fermentation

The meta-analysis study demonstrated that HMT led to notable alterations in rumen fermentation parameters, as shown in Table 3. No significant effect was observed on pH (estimate = 0.275, p = 0.365), but $NH_3$ concentration decreased significantly (estimate = -0.683, p = 0.022), indicating a reduction in ammonia production during fermentation. Total VFA concentrations showed a significant decrease (estimate = -1.634, p < 0.001), with acetate levels showing a non-significant increase (estimate = 0.450, p = 0.157), while both propionate and butyrate levels significantly declined (estimate = -1.621, p < 0.001 and -0.937, p = 0.004, respectively). Furthermore, the acetate-to-propionate ratio increased significantly (estimate = 1.545, p < 0.001), suggesting a shift towards greater acetate production. Total gas production after 48 hours also saw a significant reduction (estimate = -1.316, p < 0.001), whereas $CH_4$ production remained statistically unchanged (estimate = 0.353, p = 0.274). These results indicate that heat moisture treatment of feed induces significant changes in rumen fermentation by lowering VFA production, altering the fatty acid profile, and potentially influencing both fermentation efficiency and methane emissions.

**Table 3.** Result of meta-analysis on in vitro rumen fermentation

| Parameter | NC | Estimate | Lower | Upper | SE | p-value |
|---|---|---|---|---|---|---|
| pH | 7 | 0.275 | -0.321 | 0.871 | 0.304 | 0.365 |
| $NH_3$ (mM) | 7 | -0.683 | -1.265 | -0.100 | 0.297 | 0.022 |
| Total VFA (mmol/dl) | 8 | -1.634 | -2.327 | -0.942 | 0.353 | <0.001 |
| Acetate (mmol/dl) | 7 | 0.450 | -0.174 | 1.075 | 0.319 | 0.157 |
| Propionate (mmol/dl) | 7 | -1.621 | -2.433 | -0.809 | 0.414 | <0.001 |
| Butyrate (mmol/dl) | 7 | -0.937 | -1.576 | -0.297 | 0.326 | 0.004 |
| Acetate:Propionate | 7 | 1.545 | 0.825 | 2.265 | 0.367 | <0.001 |
| Total gas 48 h (ml) | 6 | -1.316 | -1.998 | -0.634 | 0.348 | <0.001 |
| $CH_4$ 48 h (ml) | 6 | 0.353 | -0.279 | 0.985 | 0.322 | 0.274 |

$NH_3$= ammonia; VFA= volatile fatty acid; $CH_4$= methane; NC= number of comparison; SE= standard error.

Reducing feed degradability in the rumen can alter the microbial ecosystem, thereby improving feed conversion efficiency in ruminants. In this study, we observed no significant changes in ruminal pH. However, a notable decrease in ammonia concentration was observed, similar to the results obtained in a meta-analysis of starch modification using organic acids in ruminant diets [18]. This reduction in ammonia can be attributed to decreased protein degradation caused by HMT processing, which facilitates protein bypass. As a result, less substrate is available for rumen microbes to produce ammonia through protein degradation [19]. The decrease in ammonia levels is also linked to a reduction in total VFA production in the rumen. HMT processing reduces feed digestibility in the rumen, while microbial populations rely on readily fermentable carbohydrates, such as starch, for growth and reproduction, both essential for protein and ammonia production. This aligns with the observed decrease in total gas production over 48 hours. The lower gas production is associated with a reduced microbial degradation rate as a result of HMT processing.

Furthermore, methane production in this study showed no significant difference between HMT-treated feed and the control. The potential reduction in methane emissions from heat-treated feed may be due to altered fermentation pathways in the rumen, which decrease the availability of hydrogen for methanogens. Another mechanism could be the modification of rumen microbial populations due to HMT processing, which lowers the activity of hydrogen-producing bacteria and increases the competition for hydrogen, ultimately reducing methane production [7]. However, there are limitations to our study, as only two papers report on methane parameters, resulting in a small sample size. Therefore, further research is necessary to explore the correlation between HMT-treated feed and methane production to provide more comprehensive conclusions.

### 3.3 Rumen degradability

The meta-analysis results on nutrient rumen degradability, as shown in Table 4, provide insights into the effects of heat moisture treatment on key degradability parameters. DMD significantly decreased (estimate = -1.090, $p < 0.001$), indicating that heat moisture treatment reduces the efficiency of dry matter breakdown in the rumen as shown in Figure 1. Similarly, OMD was significantly reduced (estimate = -1.271, $p < 0.001$). In contrast, crude protein degradability (CPD) showed a non-significant increase (estimate = 0.565, $p = 0.238$). These findings suggest that heat moisture treatment has a more pronounced negative impact on dry and organic matter degradability, while its effect on crude protein degradability remains unclear.

**Table 4.** Results of meta-analysis on nutrient rumen degradability

| Parameter | NC | Estimate | Lower | Upper | SE | P-value |
|---|---|---|---|---|---|---|
| DMD (%) | 22 | -1.090 | -1.160 | -1.020 | 0.209 | <0.001 |
| OMD (%) | 19 | -1.271 | -1.680 | -0.861 | 0.209 | <0.001 |
| CPD (%) | 6 | 0.565 | -0.373 | 1.504 | 0.479 | 0.238 |

DMD= dry matter degradability; OMD= organic matter degradability; CPD= crude protein degradability; NC= number of comparison; SE= standard error.

The lower DMD and OMD in heated feed are associated with heat-induced production of protein-carbohydrate matrix polymers. This is reflected in the increase of acid detergent insoluble protein and neutral detergent indigestible protein [6]. The high temperatures during the HMT process can disrupt protein-carbohydrate cross-linkages and denature molecular structures. Moreover, Nagy et al. [20] reported that heat treatment significantly affects food properties, such as strength, elasticity, and leaching losses, due to interactions between carbohydrates and proteins, as well as between carbohydrates themselves. This is generally followed by an increase in crystallinity or a more compact structure [15], which makes the feed less accessible to microbial and enzymatic digestion. In contrast, CPD values remain statistically unchanged. This is due to the bypass effect of HMT-treated protein in the rumen, where it is subsequently digested in the intestine, significantly enhancing animal production efficiency [21]. This finding aligns with Castro et al. [22], who observed that changes in protein degradation pathways occur without significantly altering total protein digestibility.

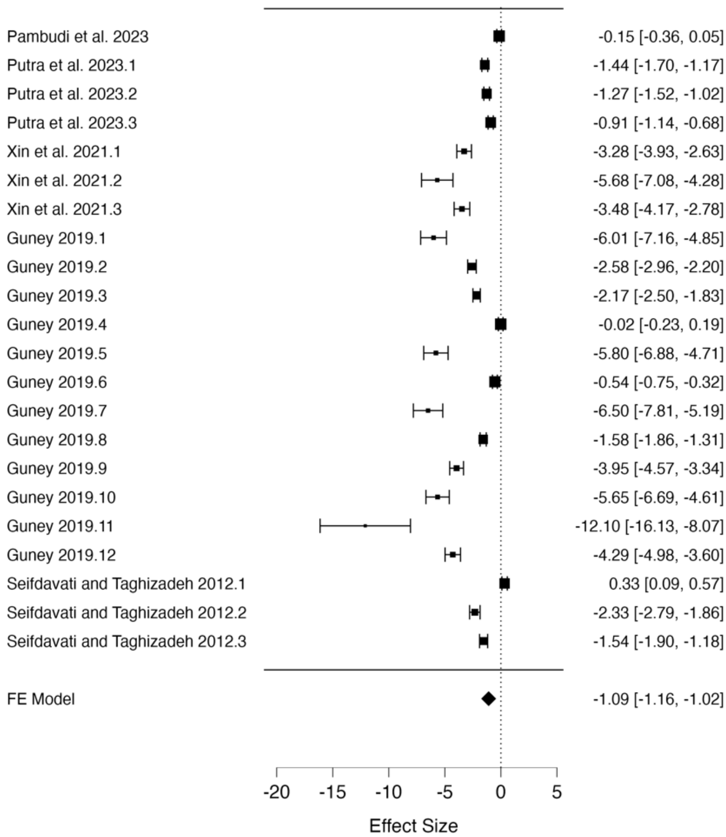


**Fig. 1.** Forrest plot of meta-analysis on dry matter rumen degradability in vitro fermentation

## 3.4 Publication bias

The evaluation of publication bias for DMD in in vitro rumen fermentation, as depicted in Figure 2, revealed potential signs of bias through the funnel plot. The statistical assessment, performed using Egger's test with a 95% confidence interval, indicated significant asymmetry ($z=28.586$, $p < 0.001$), suggesting the presence of publication bias within the data. To address this bias, the trim-and-fill method by Duval and Tweedie [23] was applied to estimate the number of missing studies. Figure 2B, with unfilled dots, demonstrates the overall effect, indicating a more symmetrical distribution of effect sizes. This suggests that, after applying the trim-and-fill method, the data may be free from publication bias.

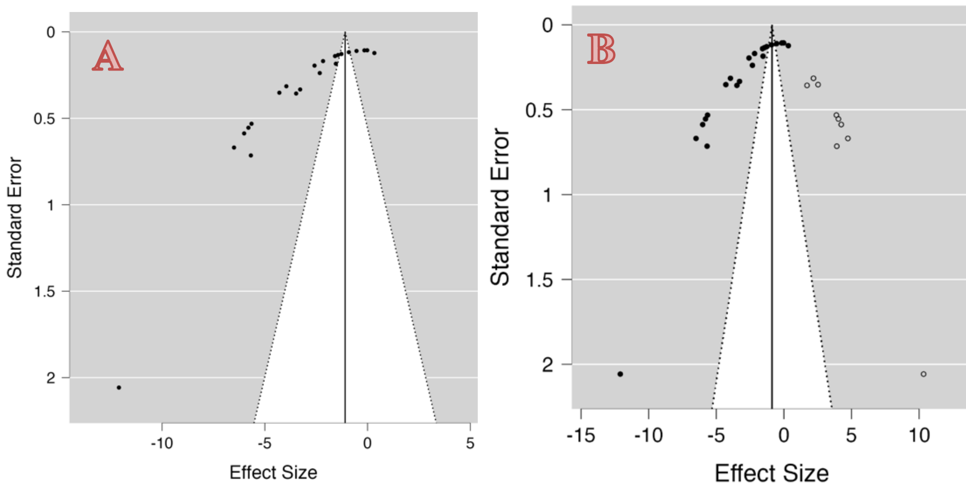


**Fig. 2.** Funnel plot of publication bias on dry matter degradability in vitro fermentation. (A) original plot, (B) after applying the trim-and-fill method.

## 4 Conclusions

In conclusion, HMT effectively modifies rumen fermentation by reducing VFAs and ammonia production, which enhances rumen health and stability, potentially boosting productivity and reducing the risk of metabolic disorders in ruminants. Its capacity to lower methane emissions also aligns with environmental sustainability objectives. Moreover, HMT allows the rumen to focus on digesting high-fiber feed, while simple but essential proteins and starch bypass the rumen for direct absorption in post-rumen organs. However, these advantages come with trade-offs, as HMT reduces the digestibility of dry and organic matter, potentially limiting nutrient availability and feed efficiency. While HMT shows promise in improving rumen metabolism and mitigating methane emissions, further research is essential to optimize its application, address these limitations, and assess its long-term effects on methane reduction and ruminant productivity.

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