







Retention of H_3PO_4 and NH_4OH by the fibrous components of oat (*Avena sativa* L.) forage

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ABSTRACT

Objective: To evaluate the structural modification of oat straw through chemical treatment with phosphoric acid (H_3PO_4) and ammonium hydroxide (NH_4OH), focusing on fiber retention and hydrolysis over time, due to its relevance as a forage resource.

Design/Methodology/Approach: Oat straw samples (500 g) were treated with 1 M aqueous solutions of H_3PO_4 and NH_4OH separately and exposed for 0, 8, 16, 24, 32, 40, and 48 hours. Samples were not washed prior to analysis to preserve any residual chemical retention on the fiber surface. The following variables were evaluated: neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, lignin (ADL), silica, and hemicellulose. Data were statistically analyzed using ANOVA and Tukey's test ($p < 0.05$). Results: After 8 hours of treatment, H_3PO_4 showed higher retention on structural components, with NDF, ADF, ADL, cellulose, and silica retaining 9.98%, 8.84%, 1.87%, 7.65%, and 2.00%, respectively. In contrast, NH_4OH treatments resulted in lower retention: 4.01% in NDF, 4.84% in ADF, and 4.11% in cellulose. Significant reductions in NDF, ADF, and cellulose content were observed with H_3PO_4 after 16-48 hours, indicating a hydrolytic effect. NH_4OH treatments did not result in significant degradation.

Study Limitations/Implications: The study did not consider the effects of post-treatment washing or neutralization, which could influence the practical application of the treated forage. Nevertheless, the findings highlight the relevance of chemical selection and exposure time in modifying lignocellulosic structures.

Findings/Conclusions: Phosphoric acid (H_3PO_4) exhibited a greater affinity and reactivity with the lignocellulosic matrix of oat straw compared to ammonium hydroxide (NH_4OH), as evidenced by higher initial retention and significant hydrolysis of structural fibers. These results suggest that H_3PO_4 may be a more effective agent for enhancing the nutritional accessibility of oat straw in ruminant diets. Further research is needed to evaluate its practical viability and safety in forage treatment systems.

Keywords: retention, phosphoric acid, ammonium hydroxide, oat straw, cell walls.

Citation: Sosa-Montes, E., Barrera-Paulino, G., Monter-Juarez, J., Borges-Filho, M.Q., Zárate-Contreras, D., Alejos-De la Fuente, J.I., Sánchez-Olguín, J., Sosa-Montes, E., & Bello-Olivera, B. (2026). Retention of H_3PO_4 and NH_4OH by the fibrous components of oat (*Avena sativa* L.) forage. *Agro Productividad*. <https://doi.org/10.32854/q9zhkg32>

Academic Editor: Jorge Cadena Iñiguez

Associate Editor: Dra. Lucero del Mar Ruiz Posadas

Guest Editor: Juan Francisco Aguirre Medina

Received: June 25, 2025.

Accepted: December 20, 2025.

Published on-line: March XX, 2026.

Agro Productividad, 19(1), January. 2026. pp: 101-111.

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INTRODUCTION

In Mexico, oats rank fifth among the crops with the largest cultivated area (Espitia *et al.*, 2007). It is a winter crop cultivated both for forage (De la Garza-Caballero &



Garza-Cedillo, 2013) and grain production (Villaseñor *et al.*, 2019; Rodríguez-Herrera *et al.*, 2020). As a food source for humans, oat grain is notable for its high dietary fiber content, primarily composed of non-starch polysaccharides, with hemicelluloses being predominant (Nowakowski *et al.*, 2008; Miller & Fulcher, 2011; Sargautiene *et al.*, 2018). These fiber fractions contribute functional and health-promoting properties, largely due to their arabinoxylan content, which can reach up to 21% (Couture *et al.*, 2022). From a forage perspective, oats are characterized by a high proportion of fibrous components such as neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose, and crude protein (Mendoza-Pedroza *et al.*, 2021). Reported values vary according to cutting stage and environmental conditions; on average, NDF ranges from 50 to 65%, and ADF from 30 to 45% (Martínez *et al.*, 2010; Coblenz *et al.*, 2013; Mendoza-Pedroza *et al.*, 2021). These lignocellulosic fractions play a key role in determining forage digestibility and nutrient retention capacity. Several studies have shown that certain plant species can absorb inorganic ions such as ammonium (NH_4^+) and phosphate (PO_4^{3-}). For instance, *Eichhornia crassipes*, *Lemna minor*, and *Saccharum officinarum* have demonstrated ion retention capabilities and are commonly employed in wastewater remediation (Raimbekov *et al.*, 2021; De Marchi *et al.*, 2022). In oats, the xylan fraction can also bind phosphate groups, forming phosphorylated polysaccharides with varying degrees of substitution (Dace *et al.*, 1997; Salama *et al.*, 2014; Laffargue *et al.*, 2023). Under acidic conditions, phosphate ions readily penetrate barley and maize roots, promoting complex formation within the cell wall (Barrow, 2017). However, limited information is available regarding the capacity of oat Straw an inert lignocellulosic residue to retain or absorb ammonium ions post-harvest. This knowledge gap is particularly relevant, as ammonium may interact with functional groups in lignin and cellulose, potentially modifying the fiber's structure and affecting its suitability for animal feed or nutrient recycling. Investigating these interactions is essential for determining whether chemical treatments with NH_4OH or H_3PO_4 differentially alter fibrous fractions, thereby influencing their composition and reactivity. Previous studies have reported that both phosphoric acid and ammonium hydroxide can act as hydrolytic agents, capable of altering structural components of the plant cell wall (Berger *et al.*, 1981; Fithri *et al.*, 2022). Prior to hydrolysis, H_3PO_4 may penetrate plant tissues and form covalent bonds with polysaccharides, whereas NH_4OH may form reversible ionic interactions (Laffargue *et al.*, 2023). Based on this, it is hypothesized that the application of H_3PO_4 and NH_4OH to oat straw results in the initial retention of phosphate or ammonium ions within fibrous fractions, followed by a progressive hydrolysis of lignocellulosic components over time.

It is expected that oat straw treated with H_3PO_4 will retain a greater amount of phosphate ions compared to NH_4OH treatment and that phosphoric acid will induce a more pronounced hydrolytic effect on lignocellulosic fractions. Therefore, this study aims to evaluate the changes in the fibrous composition of oat straw treated with H_3PO_4 or NH_4OH over a 48-hour period and to determine the retention and hydrolytic effects of both compounds.

MATERIALS AND METHODS

Experimental site

The study was conducted at the Animal Nutrition Laboratory, Department of Zootechnics, located on Peripheral 5 of the Universidad Autonoma Chapingo, Texcoco de Mora, State of Mexico (19° 29' 23" N, 98° 53' 30" W). According to García (2004), the regional climate is classified as C(w2)(w)b(i)g a temperate sub-humid climate, the driest among the sub-humid types with a mean annual temperature of 15.2 °C and an average annual precipitation of 636.5 mm, primarily occurring in the summer.

Biological material and handling

The experiment was conducted between April and July 2023 using oat straw (*Avena sativa* L.), Chihuahua variety, harvested at the pre-flowering stage from experimental plots at the Colegio de Postgraduados (COLPOS), Montecillo Campus, Texcoco, Mexico (19° 27' 51" N, 98° 54' 21" W; 2,252 masl). The straw was baled (average weight ≈36 kg) and stored under a corrugated galvanized metal roof on a concrete floor to prevent moisture exposure. A 2 kg subsample was taken to the Animal Nutrition Laboratory for immediate analysis.

Chemical analysis

All analyses were performed at the Animal Nutrition Laboratory, Department of Zootechnics, Universidad Autónoma Chapingo. The fibrous components of the lignocellulosic matrix were determined following the methodology proposed by Van Soest (1991). Each sample constituted one experimental unit and was analyzed in triplicate.

Solutions and treatments

An untreated control (0 h) was included as a baseline. Analytical-grade ammonium hydroxide (27.3% purity, density 0.88 g/cm³) and phosphoric acid (85% purity, density 1.88 g/cm³) were used. Both solutions were diluted to a concentration of 1 M using distilled water. For each treatment, 500 g of oat straw was placed in plastic bags to ensure uniform mixing and manually sprayed with the respective solution for homogeneous distribution. The treated samples were sealed and incubated at room temperature for 0, 8, 16, 24, 32, 40, and 48 hours before analysis (Van Soest, 1991).

Evaluated variables

Based on the Van Soest (1991) methodology, the following response variables were measured: neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), cellulose, silica, and hemicellulose (Hem).

Determination of NDF and its fractions

Crude fiber determination underestimates the cell wall content in forages; therefore, Van Soest's method uses a neutral detergent solution to isolate the cell wall (NDF), which comprises lignin, cellulose, hemicellulose, and silica. ADF is the residue after acid detergent digestion, where hemicellulose is removed. Subsequent treatment of ADF with sulfuric

acid (H_2SO_4) dissolves cellulose, leaving lignin and silica, which are separated via ashing at approximately 500 °C. The difference between 100 and NDF represents the cell contents, which are considered 98% digestible. NDF is partially digestible by ruminants, and its digestibility can be improved with acid or alkaline treatments.

Experimental design

A completely randomized design with a factorial treatment structure was employed: two levels of chemical treatment (H_2PO_4 and NH_4OH) \times seven exposure times (0, 8, 16, 24, 32, 40, and 48 hours). In addition to the two-way factorial analysis, each chemical compound was also analyzed independently using a one-way completely randomized design. The 48-hour maximum exposure period was selected based on preliminary trials and prior studies, indicating that measurable hydrolytic or retention effects of H_2PO_4 and NH_4OH occur within this timeframe (Krongtaew *et al.*, 2010; Sabir *et al.*, 2020). Longer durations may lead to excessive fiber degradation or reagent loss through evaporation, potentially compromising data consistency.

Statistical analysis

Data were analyzed using the general linear model procedure (SPSS, 2011). Significance was assessed at $p < 0.05$ for main effects and interactions within the factorial arrangement. The main effect of chemical compound and the interaction term were evaluated using the F-test ($p < 0.05$), while differences in the time factor were analyzed using Tukey's test ($p < 0.05$). Additionally, the effect of time for each chemical treatment was analyzed separately using Tukey's test ($p < 0.05$). No data transformations were required. Each experimental unit consisted of the mean of three replicates derived from a single straw batch per treatment and time point.

RESULTS AND DISCUSSION

Main effects and interaction

The results of the factorial analysis, including the main effects of time and chemical compound, as well as their interaction, are presented below. The time factor consisted of seven levels (0, 8, 16, 24, 32, 40, and 48 hours), while the chemical compound factor included two levels (H_3PO_4 and NH_4OH). These effects are summarized in Tables 1 and 2, with their interaction illustrated in Figure 1.

Table 1. Main effects of the chemical compounds (H_3PO_4 and NH_4OH) on fiber fractions of oat straw (% dry matter).

Chemical compound	NDF	ADF	ADL	Cellulose	Silica	Hem
H_3PO_4	64.05	38.55b	4.42b	31.43b	4.68b	25.50a
NH_4OH	64.18	42.84a	5.38a	34.21a	5.69a	21.33b
EE	0.2081	0.3273	0.0713	0.2535	0.0754	0.3467
P value	0.6670	<0.01	<0.01	<0.01	<0.01	<0.01

*NDF=neutral detergent fiber; ADF=acid detergent fiber; ADL=acid detergent lignin; Hem=hemicellulose; SE=standard error.

Means for each chemical compound, irrespective of exposure time, were compared using an F-test ($p < 0.05$). Different letters within the same column indicate significant differences.

Table 2. Main effects of exposure time (0, 8, 16, 24, 32, 40, and 48 h) on fiber fractions of oat straw (% dry matter), regardless of chemical compound.

Time (h)	NDF	ADF	ADL	Cellulose	Silica	Hem
0	58.48c	35.14b	3.81b	28.55c	4.02b	23.34
8	65.98a	42.43a	5.19a	34.43a	5.46a	23.55
16	65.50a	42.48a	5.27a	34.24ab	5.55a	23.02
24	64.70ab	41.26a	4.83a	33.49ab	5.11a	23.44
32	65.19ab	41.84a	5.34a	33.48ab	5.66a	23.36
40	65.45a	41.18a	4.89a	33.31ab	5.19a	24.27
48	63.51b	40.54a	4.98a	32.25b	5.29a	22.96
EE	0.3893	0.6123	0.1334	0.4742	0.1411	0.6486
P value	<0.01	<0.01	<0.01	<0.01	<0.01	0.8430
0	58.48c	35.14b	3.81b	28.55c	4.02b	23.34

*NDF=neutral detergent fiber; ADF=acid detergent fiber; ADL=acid detergent lignin; Hem=hemicellulose; SE=standard error. All data were compared across time using Tukey’s test ($p < 0.05$), regardless of chemical compound. Different letters within the same column indicate significant differences.

NDF remained unchanged ($p > 0.05$), while Hem content was higher and the other variables were lower with phosphoric acid (H_3PO_4) compared to ammonium hydroxide (NH_4OH). According to the results presented in Table 1, ADF, ADL, cellulose, and silica retained more NH_4OH than H_3PO_4 , whereas Hem retained more H_3PO_4 than NH_4OH .

The Hem variable remained unaffected by the time factor, while NDF and cellulose increased up to 8 h (indicating a retention effect) and decreased by 48 h, with this decline interpreted as a hydrolytic effect. The remaining response variables increased until 8 h and then remained stable through 48 h (Table 2). These results are further examined below, and Tables 3 and 4 detail the effects of time for each level of the chemical compound factor (H_3PO_4 and NH_4OH). Figure 1 illustrates the interaction between compound and time, revealing two distinct phenomena: (1) an initial retention phase (increase at 8 h),

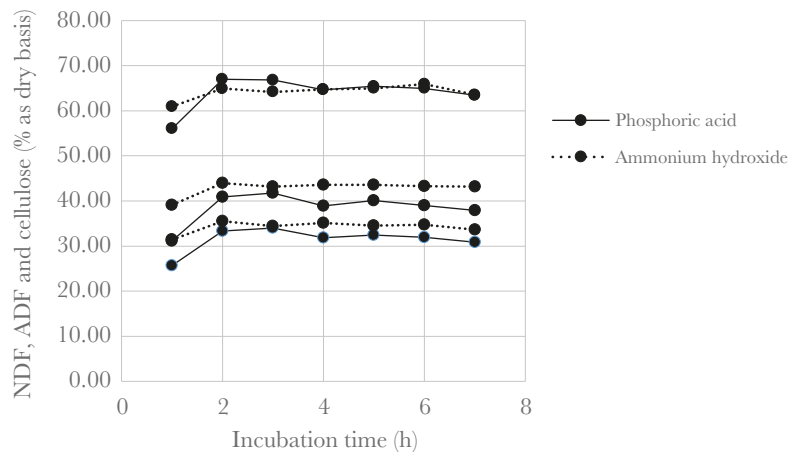


Figure 1. Interaction between chemical compound (H_3PO_4 and NH_4OH) and exposure time on neutral detergent fiber (NDF), acid detergent fiber (ADF), and cellulose content of oat straw. Solid lines represent H_3PO_4 and dashed lines represent NH_4OH . Significant compound \times time interactions were detected ($p < 0.05$).

during which H_3PO_4 binds to the fiber matrix, and (2) a hydrolytic phase (decline from 16 to 48 h), where phosphate progressively cleaves glycosidic and ester bonds. In contrast, NH_4OH exhibits only the retention phase, indicating a limited hydrolytic capacity. This distinction holds practical significance, as it suggests that acid and alkaline treatments can be strategically applied depending on whether the goal is nutrient enrichment or fiber degradation.

Subsequently, no further changes were observed, as the trend lines for each variable remained parallel. The greater initial increase with phosphoric acid suggests that NDF, ADF, and cellulose exhibit a higher affinity for phosphate retention compared to ammonium.

The interaction between the factors time and chemical compound was significant for neutral detergent fiber (NDF), acid detergent fiber (ADF), and cellulose ($p < 0.05$). No significant interactions were observed for the other response variables ($p > 0.05$). In Figure 1, the upper pair of lines corresponds to NDF: solid line (H_3PO_4) and dashed line (NH_4OH); the middle pair to ADF: solid line (H_3PO_4) and dashed line (NH_4OH); and the lower pair to cellulose: solid line (H_3PO_4) and dashed line (NH_4OH). *Eichhornia crassipes* and *Lemna minor* are known to absorb phosphates (Pavlidis *et al.*, 2022), which is why they are commonly used in wastewater treatment. Sugarcane also takes up phosphate ions, with De Marchi *et al.* (2022) reporting peak phosphate absorption in sugarcane leaves at 49 days post-transplantation. Sabir *et al.* (2020) demonstrated that cellulose phosphate or cellulose/hydroxyapatite can form at room temperature. According to Barrow (2017), phosphate uptake by isolated barley or maize roots is notably high at pH 4. Hilt *et al.* (2016) observed that oats can absorb excess phosphate from manure. Furthermore, sodium trimetaphosphate has been shown to crosslink starch and carboxymethyl cellulose, forming hydrogels (Cagnin *et al.*, 2021; Marim *et al.*, 2024). Therefore, it is considered that in the present study, the phosphate ion reacted with the oat straw sample under low pH conditions and was not removed by the neutral detergent reagent at pH 7. Consequently, NDF values at 8 h were higher than at 0 h, as were those of ADF, ADL, cellulose, and silica (Table 2). A greater retention of phosphate than ammonium has also been reported for rice straw. This higher phosphate retention suggests that rice straw carries more positive than negative charges (Sooksawat *et al.*, 2021). Additionally, the lignocellulosic matrix of straw, characterized by its porous structure and surface charges, allows for electrostatic interactions that enhance ion retention (Marim *et al.*, 2024). Polysaccharides such as cellulose and hemicellulose, rich in hydroxyl groups, can form hydrogen bonds with these ions, while lignin, with its complex and heterogeneous polymeric structure, offers additional adsorption sites. These combined interactions contribute to the capacity of oat straw to retain phosphate and ammonium ions.

Effect of phosphoric acid on oat straw over time

With H_3PO_4 , NDF was higher at 8 h of treatment compared to 0 h, and consequently, ADF, ADL, cellulose, and silica also increased. After 8 h, NDF decreased, and when comparing 48 h and 16 h, NDF, ADF, and cellulose were lower at 48 h (Table 3), indicating a tendency toward hydrolysis as the exposure time to phosphoric acid increased. It is likely

Table 3. Effects of phosphoric acid (H_3PO_4) exposure time on fiber fractions of oat straw (% dry matter).

Time (h)	NDF	ADF	ADL	Cellulose	Silica	Hem
0	56.02c*	31.16c	2.88d	25.68c	3.05d	24.86
8	66.00a	40.90ab	4.75abc	33.33ab	5.05abc	26.10
16	66.83a	41.76a	4.95ab	34.00a	5.22ab	25.06
24	64.67ab	38.94ab	4.37c	31.84ab	4.64c	25.73
32	65.41ab	40.11ab	4.96a	32.41ab	5.25a	25.30
40	64.97ab	39.06ab	4.47bc	31.92ab	4.72bc	25.91
48	63.45b	37.89b	4.55abc	30.82b	4.80abc	25.56
EE	0.5255	0.7183	0.1013	0.6064	0.1071	0.8757
P value	<0.01	<0.01	<0.01	<0.01	<0.01	0.9431

*NDF=neutral detergent fiber; ADF=acid detergent fiber; ADL=acid detergent lignin; Hem=hemicellulose; SE=standard error. Means within each column were compared using Tukey's test ($p < 0.05$). Different letters within the same column indicate significant differences.

that H_3PO_4 is retained primarily by cellulose and NDF, rather than by other fractions. Krongtaew *et al.* (2010) reported that the total polysaccharides in oat and wheat straw increased after 4 h of acid (pH 3, 25 °C) or alkali (pH 11.5, 90 °C) treatment. In oat straw, the total polysaccharides rose from 51% to 71.5% with acid and to 74% with alkali treatment. When oat and wheat straws were treated with sulfuric acid (pH 3, 25 °C, 4 h), Krongtaew *et al.* (2010) observed increases in total polysaccharides of 20.5% and 4.4%, respectively. These findings align with those of Sabir *et al.* (2020), who demonstrated that at room temperature, cellulose phosphate or cellulose/hydroxyapatite complexes can form. Sodium trimetaphosphate is also capable of crosslinking starch and carboxymethyl cellulose to produce hydrogels (Cagnin *et al.*, 2020; Marim *et al.*, 2024). It is important to note that the studies by Krongtaew *et al.* (2010) and Sabir *et al.* (2020) employed more extreme conditions (acidic pH 3-4, temperatures up to 90 °C, and short reaction times of 4 h), whereas the present study was conducted at room temperature and moderate acidity (1 M H_3PO_4). These methodological differences account for the milder hydrolytic effects observed in this experiment. No additional data were found regarding phosphate retention by the other lignocellulosic fractions. While the uptake of ammonium and phosphate ions by living plants has been well documented due to the relevance of fertilization (Sharma & Chetani, 2017), the retention of these ions by post-harvest inert materials such as oat straw has received limited attention. Research has instead focused on the hydrolysis of lignocellulosic components. This hydrolytic effect is addressed in the following discussion. After 8 and 16 h of treatment, phosphoric acid begins to exhibit hydrolytic activity, resulting in lower levels of NDF, ADF, and cellulose at 48 h compared to 16 h (Table 3). However, the other variables remained unchanged. It is possible that beyond 48 h, this reduction could become more pronounced. Since the present study was limited to a maximum of 48 h, any further decline beyond this point was not observed in comparison to the original (0 h) sample. These findings suggest that phosphate ions infiltrate the cell wall and form stable phosphate esters with the hydroxyl groups of cellulose and hemicellulose, leading to increased apparent retention. In contrast, ammonium ions (NH_4^+) interact primarily

through ionic exchange with negatively charged sites, which are relatively scarce in lignocellulosic materials, thereby explaining their lower retention capacity.

Effect of ammonium hydroxide on oat straw over time

The lack of significant changes in most variables with NH_4OH may be attributed to the lower reactivity of ammonium ions compared to phosphate ions. Ammonium tends to interact electrostatically with lignin and phenolic groups, forming weak, reversible bonds that do not disrupt the fiber matrix. Thus, under the tested conditions (1 M, room temperature), NH_4OH acts primarily as a mild alkalizing agent rather than a potent hydrolytic compound.

With NH_4OH , an increase in NDF, ADF, and cellulose levels was observed between 0 and 8 h, with no further changes ($p > 0.05$). The variables ADL, silica, and Hem were unaffected by NH_4OH treatment. Not a single variable showed a decrease after 8 or 16 h of treatment (Table 4). However, at extended treatment durations, NH_4OH could exhibit hydrolytic effects. For instance, Berger *et al.* (1981) reported increased digestibility of oat kernels treated with 6% NaOH and 6% NH_4OH over three weeks, attributing the improvement to hemicellulose solubilization. Similarly, Zhang *et al.* (2018) demonstrated high glucose yields from sugarcane bagasse through sulfuric acid hydrolysis followed by ethanol/NaOH treatment. When oat and wheat straws were treated with sodium hydroxide (pH 11.5, 90 °C, 4 h), Krongtaew *et al.* (2010) observed increases in total polysaccharides of 11.1% and 23%, respectively. Bachmann *et al.* (2023) also reported a 6.3% increase in ash content when barley straw was treated with NaOH (6 g/kg DM, 5 min), rewetted to 40% dry matter, and stored for 14 days. In Krongtaew *et al.*'s study, the increases were likely due to a concentration effect following distilled water washing a step not performed in the present study. An increase in NDF content over time was observed, with phosphoric acid resulting in a 9.98% rise (Table 3), significantly greater ($p > 0.05$) than the 4.01% increase with ammonium hydroxide (Table 4). This was not due to a concentration effect, as no sample washing occurred before analysis. Hence,

Table 4. Effects of ammonium hydroxide (NH_4OH) exposure time on fiber fractions of oat straw (% dry matter).

Time (h)	NDF	ADF	ADL	Cellulose	Silica	Hem
0	60.94b*	39.12b	4.73	31.41b	4.98	21.82
8	64.95a	43.96a	5.62	35.52a	5.88	21.00
16	64.18a	43.20ab	5.59	34.48ab	5.88	20.97
24	64.72a	43.57ab	5.29	35.15a	5.58	21.15
32	64.97a	43.56ab	5.72	34.54ab	6.07	21.41
40	65.93a	43.30ab	5.32	34.70ab	5.66	22.63
48	63.56ab	43.19ab	5.41	33.68ab	5.78	20.36
EE	0.5744	0.9919	0.2468	0.7293	0.2612	0.9569
P value	<0.01	0.0520	0.1760	0.0252	0.1630	0.7476

*NDF=neutral detergent fiber; ADF=acid detergent fiber; ADL=acid detergent lignin; Hem=hemicellulose; SE=standard error. Means within each column were compared using Tukey's test ($p < 0.05$). Different letters within the same column indicate significant differences.

phosphoric acid was more strongly retained by NDF than ammonium hydroxide. Since NDF includes cellulose, this result aligns with findings by Marim *et al.* (2024), who showed that sodium trimetaphosphate can bind to cellulose chains. From a practical standpoint, understanding the differential retention and hydrolysis patterns of H_3PO_4 and NH_4OH in oat straw can inform strategies to enhance forage digestibility. Mild acid pretreatments such as H_3PO_4 may partially solubilize hemicellulose and improve fiber accessibility to microbial enzymes, thereby promoting ruminal degradation. Conversely, NH_4OH treatments could be more suitable when the objective is to boost nitrogen content without causing significant structural breakdown.

CONCLUSIONS

The evaluation of the retention and hydrolytic effects of phosphoric acid (H_3PO_4) and ammonium hydroxide (NH_4OH) on the fibrous fractions of oat straw was successfully carried out. The results showed that both compounds interacted with the lignocellulosic matrix, though with differing intensities and mechanisms. Phosphoric acid exhibited significantly greater retention and hydrolytic activity ($p < 0.05$) than ammonium hydroxide, as demonstrated by the more pronounced reductions in NDF, ADF, and cellulose contents after 16-48 h of treatment. These findings confirm that phosphate ions form stronger chemical bonds with polysaccharides, while ammonium ions primarily engage in weak electrostatic interactions. The retention observed at 8 h and the hydrolysis noted at 48 h represent two distinct chemical processes: initial ion adsorption followed by partial depolymerization of the fiber matrix. From an applied perspective, these results suggest that mild phosphoric acid treatments could enhance the digestibility of oat-based forages through partial disruption of lignocellulosic bonds, while ammonium hydroxide could serve to enrich nitrogen content without damaging fiber structure. Future studies should examine the *in vivo* effects of these treatments on ruminal fermentation and nutrient bioavailability.

ACKNOWLEDGMENTS

To our honorable and beloved Universidad Autónoma Chapingo for opening its doors to us, as well as to the Animal Nutrition Laboratory of the Department of Zootechnics and its dedicated team for their support during the experimental phase of this research project.

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