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Valorization of Date Pedicels via Urea Treatment and Solid-State Fermentation: Effects on Nutritional Composition and *In Vitro* Digestibility for Ruminants

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Abstract

Date pedicels (DP), an abundant by-product of date palm cultivation, are underutilized in animal feeding systems due to their high lignocellulosic content. This study aimed to enhance the nutritional value and digestibility of DP through urea treatment and solid-state fermentation (SSF) using white-rot fungi. The effects of these treatments on chemical composition, fiber fractions, and in vitro gas production were evaluated.

DP were treated with urea (DPU), SSF using *Fomes fomentarius* and *Lentinus tigrinus* (DPF and DPL), and their combination (DPUF and DPUL).

Crude protein (CP) content increased from 3.5% in DP to 8.5% in DPU. Moreover, SSF with F. fomentarius raised CP from 3.5% in DP to 4% in DPF and from 8.5% in DPU to 9% in DPUF ($P < 0.001$).

Both urea and SSF treatments significantly reduced neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents ($P < 0.001$). SSF was particularly effective in degrading ADF, lowering its content from 44.59% in untreated DP to 33.18% in DPF and 31.44% in DPL ($P < 0.05$). Lignin degradation was also improved, with the acid detergent lignin (ADL) fraction in DPL decreasing from 20.04% to 16.99%.

In vitro gas production increased significantly in all treated groups, especially in the co-treated substrates (DPUF and DPUL), indicating improved fermentability.

In conclusion, the combination of urea and SSF treatments represents a promising strategy for the valorization of date pedicels. This approach improves fiber degradation and enhances the nutritional and fermentative properties of the substrate, supporting its potential use in ruminant diets.

Keywords White rot fungus. Solid state fermentation. Urea. Ruminant feed. Digestibility. Date pedicels

Introduction

In Tunisia, livestock farming has expanded since the 1970s (Ben Salem, 2011). Feed costs comprise 60-80% of total production expenses (Rejeb Gharbi et al., 2007; Ben Rayana et al., 1994). However, the availability of local feed resources has not kept pace with the growth of livestock production, leading to a substantial gap between the nutritional needs of animals and actual intake (Ben Salem, 2011).

Among the underutilized agricultural by-products, the lignocellulosic biomass of date pedicels, residues from the Tunisian date palm industry, can be explored as a potential alternative animal feed resource. Nevertheless, their use is limited by their high content of structural carbohydrates and lignin, which form complex matrices that reduce biodegradability and digestibility (Brodeur et al., 2011; Dinis et al., 2009).

In ruminant nutrition, fiber is essential for maintaining a healthy digestive system and supporting efficient ruminal fermentation (Varga and Kolver, 1997; Wang et al., 2022). However, the tight association of cellulose and hemicellulose within the complex lignocellulosic matrix, coupled with the resistant nature of lignin, impedes microbial degradation of carbohydrates and limits nutrient availability in the rumen. This recalcitrance reduces the overall nutritional value of fiber-rich by-products and restricts their use in ruminant diets (Van Kuijk et al., 2014; Weimer, 2022). Consequently, developing effective pretreatment methods to disrupt the lignocellulosic structure is critical to enhancing the utilization of these resources and improving ruminant feed quality.

Various pretreatment methods have been explored to disrupt the lignin–holocellulose complex and enhance the accessibility of cellulose and hemicellulose for rumen microorganisms, thereby improving digestibility (Sarnklong et al., 2010). These methods include physical, chemical, physicochemical, and biological treatments.

Physical treatments such as grinding, soaking, or pelleting generally have limited effects on the digestibility of lignocellulosic biomass (Turgut, 2008). Chemical treatments with acids (e.g., sulfuric or nitric acid) or alkalis (e.g., sodium hydroxide or potassium hydroxide) have demonstrated significant improvements in digestibility (Fahmy and Klopfenstein, 1994). However, these methods tend to be costly and pose environmental risks due to the use of harsh chemicals (Tuyen et al., 2012). In contrast, urea treatment represents a relatively inexpensive and widely applied chemical approach for crop residue processing. Urea is a non-protein nitrogen-containing compound used by ruminants as an ammonia source via rumen microbial conversion.

Physicochemical methods, including ionizing radiation, steam explosion, acid or alkali dilution, and oxidation, have also been investigated. Nevertheless, these techniques require substantial energy input, specialized equipment, and may generate toxic waste products, limiting their practical application (Hendriks and Zeeman, 2009).

Biological processes have garnered considerable interest due to their cost-effectiveness and environmentally friendly nature. Among these treatements, white rot fungi are recognized as the primary organisms capable of degrading the complex three-dimensional structure of lignin into carbon dioxide and water (Hatakka and Hammel, 2010). The fungal mycelium secretes key enzymes, including lignin peroxidase, manganese peroxidase, and laccase, which play crucial roles in breaking the bonds within lignocellulosic materials.

SSF using white rot fungi, defined as the growth of microorganisms on a moist solid substrate in the absence of free-flowing water, has proven effective in lignocellulose degradation through the production of lignocellulolytic enzymes. Recently, Abid et al. (2023) demonstrated that SSF of grape pomace waste with *Pleurotus cornucopiae* and *Ganoderma resinaceum* effectively degraded lignin compounds and improved rumen degradability.

Although urea treatment and fungal fermentation have been individually studied for improving the nutritive value of lignocellulosic biomass (Martens et al., 2022; Nayan et al., 2018), their combined application, particularly in date pedicels, remains largely unexplored. This synergistic approach has the potential to overcome the limitations associated with each treatment alone, offering a novel strategy for valorizing this underutilized by-product.

The objective of this study was to evaluate the chemical composition of DP and to assess their transformation into a digestible and nutritious ruminant feed through solid-state bioconversion using two ligninolytic fungi, *Fomes fomentarius* and *Lentinus tigrinus* (Fig.1). Urea was added as a nitrogen source to support fungal growth and enhance metabolic activity (Fig.2). Nitrogen is a critical nutrient for fungi, essential for the synthesis of proteins, nucleic acids, and other cellular components necessary for fungal development and energy production, thereby promoting efficient metabolism and colonization of the substrate (Tudzynski, 2014; Bellettini et al., 2019; Tang et al., 2024).

This study systematically compares three treatment approaches: solid-state fungal fermentation alone, urea treatment alone, and their combined application. The evaluation focuses on four key parameters: proximate composition, cell wall constituents, in vitro digestibility, and gas production kinetics. To our knowledge, this work represents the first comprehensive assessment of potential synergistic effects between chemical and biological treatments applied to this underutilized agricultural by-product.

1. Materials and methods

1.1. Substrate

DP were collected from the Gafsa region, sourced from 40- to 60-year-old Phoenix dactylifera L. trees of the 'Deglet Nour' cultivar. The orchards are traditionally managed, including the use of organic fertilization (manure), with manual date harvesting taking place between mid-October and mid-November. Pedicel collection occurred during post-harvest pruning. To ensure sample representativeness, pedicels were randomly selected from various bunches and trees across multiple orchards. After collection, samples were stored in a dry, well-ventilated room at ambient temperature until further processing.

Subsequently, the pedicels were oven-dried at 60 °C for 48 hours and then ground using a laboratory hammer mill (Werkhuizen Schepens nv) to achieve a particle size of less than 2 mm.

1.2. Microorganisms

Fomes fomentarius (MUCL 35117) and *Lentinus tigrinus* (MUCL 40499), used in this study, were obtained from the Belgian Coordinated Collections of Microorganisms / Mycothèque de l'Université Catholique de Louvain (BCCM/MUCL). Stock cultures were maintained at 4 °C on 2% malt extract agar (MEA).

For inoculum preparation, the fungi were cultured in 200 mL of 2% MEA in 500 mL Erlenmeyer flasks and incubated on a rotary shaker at 120 rpm at 28 °C for 10 days. The resulting liquid cultures were centrifuged at 4500 rpm for 15 minutes. The recovered pellets were washed three times with physiological saline (9 g NaCl/L distilled water) and homogenized using sterilized glass beads under vigorous agitation to disrupt the mycelial mass.

1.3. Experimental setup

1.3.1. Solid state fermentation

SSF was conducted in 1000 mL Erlenmeyer flasks, each containing 20 g of DP. The substrate was moistened with distilled water to achieve 50% moisture content and then autoclaved at 121 °C for 30 minutes to eliminate native microbial contaminants. Each flask was inoculated with 5 mL of fungal suspension of either *F. fomentarius* or *L. tigrinus*. The flasks were incubated at 28 °C for 22 days in a static incubator. This incubation period and temperature were selected based on preliminary trials that indicated optimal fungal colonization and are in line with previous SSF studies involving white-rot fungi (Andrade et al., 2017; Wang et al., 2023; Zuo et al., 2019). All treatments were performed in duplicate. After incubation, the fermented substrates were dried in a hot air oven at 60 °C until constant weight was achieved. Untreated DP served as the control throughout the experiment.

1.3.2. Urea treatment and urea /SSF co-treatment

DPU was carried out according to the method described by Chenost and Kayouli (1997) for total ureolysis. Briefly, DP was treated with a 4% urea solution (w/w), adjusted to 50% moisture content, and incubated anaerobically at 30 °C for 8 days. After treatment, the samples were dried and stored for further use in SSF experiments.

For the urea/SSF co-treatment, nitrogen addition was hypothesized to enhance substrate colonization. However, it is well established that lignin degradation by white-rot fungi naturally occurs in nitrogen-poor environments (Tripathi and Yadav, 1992). In fact, white-rot fungi preferentially utilize organic nitrogen sources such as urea, which can conserve cellular resources and energy but may suppress ligninolytic enzyme activity (Bautista et al., 2019).

To balance these effects and stimulate the ligninolytic potential of the fungi, DP was mixed with DPU. Preliminary tests identified a 50/50 (w/w) ratio as optimal for mycelial development. This mixture was then subjected to the same SSF conditions as previously described.

The experimental groups were as follows:

- **DP**: untreated control
- **DPU**: urea-treated DP
- **DPF**: SSF-treated with *F. fomentarius*
- **DPL**: SSF-treated with *L. tigrinus*
- **DPUF**: urea and SSF co-treatment with *F. fomentarius*
- **DPUL**: urea and SSF co-treatment with *L. tigrinus*

1.3.3. Enzyme assay

Following incubation, 1 gram of each fermented sample was homogenized with 20 mM sodium acetate buffer (pH 5) at a ratio of 10 mL buffer per gram of substrate. The mixture was shaken at 160 rpm for 1 hour at room temperature. After extraction, the suspension was centrifuged at 4500 rpm for 15 minutes, following the method of Neifar et al. (2009). Laccase activity in the supernatant was measured spectrophotometrically by monitoring the oxidation of 5 mM 2,6-dimethoxyphenol (DMP) at 469 nm for 1 minute, as described by Jaouani et al. (2005).

1.3.4. Chemical analysis

Prior to chemical analysis and in vitro gas production assays, the samples were ground to pass through a 1 mm screen using a Polymix® hammer mill. Dry matter (DM) and ash contents (% DM) were determined according to AOAC (1980) methods. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed following the procedures of Van Soest (1982, 1991). Nitrogen content was determined by the Kjeldahl method, and crude protein (CP) content (% DM) was calculated as N × 6.25, following AOAC (1990). To determine total phenolic content, 1 g of dried date palm leaf (DPL) substrate was extracted with 5 mL of methanol/water (80:20 v/v), incubated at 70 °C in a dry bath for 1 hour, and then centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected as described by Alu'datt (2010). Total phenolics were quantified using the Folin–Ciocalteu method (Folin and Ciocalteu, 1927), with gallic acid monohydrate as a standard.

For total sugar determination, 1 g of DPL substrate was mixed with 6 mL of distilled water and incubated in a 70 °C water bath for 3.5 hours with occasional shaking. The mixture was left overnight in a refrigerator, followed by centrifugation at 2,500 rpm for 10 minutes. The supernatant was collected (Bachelier and Gavinelli, 1966), and total sugar content was determined using the Anthrone reagent method (Morris, 1948).

All chemical composition results were expressed on a dry matter basis (% DM).

1.3.5. *In vitro* gas production study

Gas production tests are routinely used in feed research as gas volumes are related to both the extent and rate of substrate degradation.

In vitro gas production was determined according to the method of Menke and Steingass (1988), with slight modifications as described by El Asri (2015). Rumen inoculum was collected under controlled conditions from four freshly slaughtered, healthy male Barbarine lambs (~9 months old, ~32 kg body weight) at a local slaughterhouse. The animals had been fed a diet consisting of oat hay supplemented with a commercial concentrate in a 70:30 ratio on a dry matter basis.

Three hundred (300) mg dry weight of samples was weighed in triplicate into 50 ml calibrated infusion bottles. The rumen fluid was collected into pre-warmed thermos flask and watering and filtered through four layers of gauze and purged with CO_2.

Thereafter, the rumen fluid was added to the mineral buffer solution (1:2 v/v) and well mixed. In each infusion bottle, 30 ml of buffered rumen fluid and feed sample were taken, and the mixture was purged with CO_2. Fifty (50) ml graduated syringes are immediately injected into the bottle sand placed into the water bath at 39°C. A control containing only buffered rumen fluid was also incubated and the total gas values were corrected. Gas volumes were recorded at 0, 2, 4, 6, 8, 12, 16, 24, 36, 48, 72 and 96 h of incubation.

Three hundred milligrams (300 mg) of each dry sample were weighed in triplicate into 50 ml calibrated infusion bottles. Rumen fluid was collected in pre-warmed thermos flasks, filtered through four layers of gauze, and purged with CO_2. The filtered rumen fluid was then mixed with a mineral buffer solution in a 1:2 (v/v) ratio.

For each bottle, 30 ml of the buffered rumen fluid was added to the feed sample. The mixture was purged again with CO_2, and a 50 ml graduated syringe was immediately attached. Bottles were then incubated in a water bath at 39°C. A blank control containing only the buffered rumen fluid was also incubated, and the total gas values were corrected accordingly.

Gas volumes were recorded at 0, 2, 4, 6, 8, 12, 16, 24, 36, 48, 72, and 96 hours of incubation.

The metabolizable energy (ME) values, organic-matter degradability (OMD) contents, and volatile fatty acids (VFAs) contents were estimated:

$ME = 2.2 + 0.136 \times GP + 0.0057 \times CP$ (Menke and Steingass, 1988)

$OMD = 14.88 + 0.889 \times GP + 0.45 \times CP + 0.0651 \times Ash$ (Menke and Steingass, 1988)

$VFA = 0.0222 \times GP - 0.00425$ (Getachew, 2002)

Where: ME is metabolizable energy in MJ/kg DM;

OMD is organic matter degradability in %;

GP is the net gas production (ml) from 300 mg after 24 hours of incubation

CP is crude protein in % DM, ash in % DM;

Ash = Ash content (% DM)

1.4. Statistical analysis

All experiments were conducted in duplicate, and each sample was analyzed in triplicate. The values presented in the figures represent mean values with a standard deviation below 5%.

Data were analyzed using one-way analysis of variance (ANOVA), considering treatment as the main factor, according to the following model:

$$Yij = \mu + Ti + eij$$

Where: Yij= observed value,

μ = overall mean;

Ti = treatment effect;

eij = random error.

Differences between the means were compared by the Duncan test on the threshold of 5%. Statistical analyses were performed using SAS software (Version 9.1, SAS Institute Inc., Cary, NC, USA).

Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979):

$$y = a + b\,(1 - e^{-ct})$$

Where: y = gas production at time t (ml),

a = gas production from the immediately soluble fraction (ml),

b = gas production from the insoluble fraction (ml),

c = gas production rate constant (ml/h),

$a + b$ = potential gas production (ml),

t = incubation time (h).

2. Results and discussion

2.1. Subjective Observations

After 22 days of incubation at 28 °C, noticeable mycelial growth was observed. The mycelia of *Fomes fomentarius* and *Lentinus tigrinus* completely colonized both DP and DPU substrates (Fig. 3).

The presence of urea did not significantly affect the growth of *F.fomentarius* and *L.tigrinus*, as their development on the urea-supplemented substrate was comparable to that on the original substrate. Notably, fungal responses to urea vary considerably across species, depending on their tolerance to toxic xenobiotics. For instance, urea has been reported as the most effective nitrogen source for *Pleurotus ostreatus* under solid-state fermentation, whereas it proved to be among the least efficient for *Pleurotus pulmonarius* (Sośnicka et al., 2022).

These preliminary results set the stage for examining the enzymatic activities driving the biochemical changes induced by the treatments.

2.2. Laccase activity

F.fomentarius and *L.tigrinus* are recognized as prominent laccase-producing fungi, frequently employed in the hydrolysis of lignocellulosic biomass during fermentation processes. Laccase plays a critical role in the degradation of lignin, thereby facilitating the accessibility of cellulose and hemicellulose to rumen microorganisms. The influence of nitrogen sources on the enzymatic activity of white rot fungi varies significantly depending on the fungal species and the compound applied (Kachlishvili et al., 2006).

In the present study, the addition of urea to the culture medium resulted in a reduction of laccase activity in both fungi. For *F. fomentarius*, laccase production decreased from 1497 U L^{-1} to 1222 U L^{-1}, while in *L. tigrinus*, activity declined from 2841 U L^{-1} to 2351 U L^{-1} (Fig.4).

This modulation of enzymatic synthesis may be attributed to the interplay between nitrogen availability and the presence of easily metabolizable carbon sources, such as glucose, which promote mycelial growth. In contrast, the presence of inducers like cellulose and lignin stimulates laccase production (D'Agostini et al., 2011). Therefore, it is plausible that in media supplemented with urea, the fungi prioritize growth by utilizing the readily available nitrogen, thereby reducing the necessity for laccase synthesis. Conversely, in the absence of urea, laccase production is induced to facilitate carbon acquisition from complex substrates.

The abundant nitrogen supplied by urea activates a regulatory mechanism known as nitrogen catabolite repression, wherein fungi preferentially utilize easily accessible nitrogen sources rather than investing metabolic energy in the synthesis of energetically expensive lignin-degrading enzymes such as laccases (Hernández et al., 2015). In this context, urea functions as a preferred nitrogen source, signaling to fungal cells that the production of complex oxidative enzymes is unnecessary. This mechanism accounts for the observed decrease in laccase activity in urea-supplemented cultures compared to those without urea addition (Suryadi et al., 2022). Consistent with this observation, González Bautista et al. (2019) reported that the use of urea as a nitrogen source led to reduced laccase activity in *Pycnoporus sanguineus*.

Under nitrogen-rich conditions, fungal metabolism tends to shift toward primary growth processes, favoring biomass accumulation and nutrient assimilation. For instance, Zhang et al. (2022) demonstrated that elevated nitrogen levels promote enhanced mycelial branching in *Stropharia rugosoannulata*. Conversely, laccase production in many fungal species is commonly induced under nitrogen-limiting conditions. As observed in *P. sanguineus*, laccase synthesis was significantly C when the fungus was cultivated in a nitrogen-deficient medium (El-Batal et al., 2014).

While the suppression of laccase activity under nitrogen-rich conditions indicates a metabolic reallocation toward vegetative growth rather than lignin degradation, it is equally important to assess how these metabolic shifts affect the substrate's carbohydrate profile, particularly in terms of sugar release and cell wall breakdown.

2.3. Sugar contents

Fig. 5 illustrates that the sugar content in DP supplemented with urea was slightly higher than in untreated DP; however, this difference was not statistically significant.

Solid-state fermentation (SSF), whether applied with or without prior urea treatment, resulted in a noticeable reduction in total sugar content compared to the untreated and urea-treated controls. This decrease is primarily attributed to the fungal consumption of soluble sugars during growth and colonization.

As reported by Arantes et al. (2012) and Agustin et al. (2019), fungi secrete carbohydrate-active enzymes that degrade plant cell wall polysaccharides, particularly cellulose and hemicelluloses, into oligosaccharides and simple sugars. These breakdown products serve as energy sources to sustain fungal metabolism and development during SSF.

The consumption of soluble sugars by fungi is usually accompanied by structural modifications of the lignocellulosic matrix; therefore, the evolution of phenolic compounds was subsequently investigated.

2.4. Phenolic contents

The reduction of phenolic content during SSF is a critical factor in improving the digestibility of agricultural residues for animal feed. Non-polysaccharide components, particularly phenolic acids, are among the main inhibitors of digestibility (Asmare, 2020).

For both fungal strains, SSF treatment, whether or not preceded by urea pretreatment, led to a reduction in phenolic compounds (Fig. 6). This can be attributed to the polymerization of phenolic compounds within the DP substrate, and their gradual degradation by laccases, thus reducing their toxicity (Bautista et al., 2019). In fact, phenolic compounds, which are structurally related to lignin and its derivatives, are among the most effective inducers of laccase production (El-Batal et al., 2014).

The reduction in phenolic content was more pronounced in SSF treatments without urea pretreatment. *F. fomentarius* and *L. tigrinus* reduced the polyphenol content to 6.64 g/kg DM and 10.04 g/kg DM, respectively, for the DP substrate, compared to 8.5 g/kg DM and 11.2 g/kg DM for the DPU substrate.

This observation suggests that fungal growth in the presence of urea, which provides an accessible nitrogen source, may reduce the need for the fungi to produce ligninolytic enzymes such as laccases. As a result, enzymatic oxidation of phenolic compounds was slowed down.

The decrease in phenolic compounds and liberated sugars together contribute to the observed modifications in cell wall composition.

2.5. Cell Wall Content

The plant cell wall is a complex structure composed of three interlinked fractions: cellulose, hemicellulose, and lignin (Lee et al., 2014). The primary aim of any pretreatment method is to disrupt these bonds and increase the accessibility of cellulose and hemicellulose to rumen microflora. Table 1 summarizes the lignocellulosic composition, where cellulose typically represents the largest proportion of the cell wall, followed by hemicellulose and then lignin.

Treatment of DP with urea and/or SSF using white-rot fungi resulted in a general decline in both NDF and ADF fractions. The most pronounced reduction was observed with SSF treatment using *L.tigrinus*. Specifically, the NDF and ADF contents of DPL decreased significantly from 68.72% to 53.81% and from 44.59% to 31.44%,

respectively ($P < 0.001$). These results are consistent with those reported by Shrivastava et al. (2011), who observed similar reductions following SSF with *Pleurotus ostreatus* on wheat straw.

The observed reduction in crude fiber suggests that the fungi solubilized and utilized cell wall components as carbon sources, thereby altering the ratio of insoluble to soluble carbohydrates (Taniguchi et al., 2005).

Statistical analysis revealed no significant differences in hemicellulose content between untreated and treated DP. However, both chemical and biological treatments significantly reduced cellulose content, particularly in DPF and DPL substrates ($P < 0.01$). This finding indicates that the enzymatic breakdown of structural carbohydrates is enhanced in the absence of urea, likely due to the increased activity of both ligninolytic and cellulolytic enzymes. The degradation of insoluble carbohydrates in the substrates is primarily facilitated by extracellular enzymes secreted by white-rot fungi. These fungi possess two major types of extracellular enzymatic systems: hydrolytic enzymes, responsible for the degradation of polysaccharides such as cellulose (cellulases), and ligninolytic enzymes, which degrade lignin and cleave aromatic rings (peroxidases and phenol oxidases) (Vorlaphim, 2016).

Chemical treatment with urea reduced the ADL content from 20.04% to 17.83%. This is because urea selectively targets lignin bonds, particularly C–O–C, ester, and ether linkages, resulting in the breakdown of lignin within the lignocellulosic matrix (Datsomor, 2022). However, the combination of urea and SSF treatment did not lead to a reduction in ADL content, likely due to suppressed laccase activity in nitrogen-rich environments.

In contrast, SSF treatment alone decreased lignin content from 20% to 19% in DPF and to 17% in DPL. White-rot fungi, commonly used in solid-state fermentation, are known to degrade both lignin and cellulose simultaneously during the bioprocessing of plant materials (Okano et al., 2005). Their ligninolytic activity involves the cleavage of inter-lignol bonds and disruption of aromatic rings, enhancing fiber digestibility (Vorlaphim, 2016).

While reducing fiber fractions generally improves digestibility, excessive degradation of structural carbohydrates can be detrimental. It may lower the effective fiber content needed to support rumen motility, maintain stable pH, and sustain a healthy microbial ecosystem. Thus, optimizing fiber degradation while preserving sufficient structural integrity is key to ensuring proper rumen function and overall animal health (Stokes, 2016; Adesogan et al., 2019; Zhou et al., 2022).

The degradation of lignocellulose and the release of assimilable sugars into fungal biomass, together with available nitrogen, set the stage for examining crude protein content.

2.6. Crude protein contents

As presented in Fig. 7, CP content of untreated DP was relatively low, at 3.5%. However, treatment with urea significantly increased the CP content to 8.5% ($P < 0.001$) compared to the untreated control. This enhancement in CP levels following urea application is attributed to the absorption of ammonia and the subsequent incorporation of inorganic nitrogen into the cell wall matrix, as reported by Datsomor (2022). Additionally, Kayouli et al. (1989) noted that water and urease enzymes, naturally present in roughage, facilitate the hydrolysis of urea into ammonia and carbon dioxide, a process that requires adequate urea diffusion and fixation to ensure complete ureolysis.

SSF treatment further improved the CP content in both untreated and urea-treated DP. Specifically, *F. fomentarius* marginally increased the CP content from 3.5% to 4% in untreated DP and from 8.5% to 9% in DPU ($P < 0.001$). Indeed, Fungi are known for their capabilities in assimilating nitrogen from the substrate (Davis and Won, 2010). However, the increase in protein content may result from the bioconversion of degraded carbohydrates into fungal biomass and the release of CO_2, thereby concentrating nitrogen within the substrate (Nayan, 2018). This suggests

an improvement in microbial protein quality, given that fungal biomass is typically rich in essential amino acids (Wang et al., 2021; Yu et al., 2023), which could enhance protein availability for ruminal microorganisms.

In fact, fungal mycelia are characterized by a relatively high protein content. According to Chang and Miles (2004), 60–70% of nitrogen in fungal cells is present in the form of protein. Moreover, fungi convert both the intrinsic nitrogen in the substrate and the nitrogen provided by the ammonification agent into protein-bound nitrogen (Wan and Li, 2010).

The combined effects of enzymatic activity, structural modifications, and protein accumulation were reflected in the in vitro digestibility and gas production, providing a comprehensive assessment of substrate utilization and nutritional quality.

2.7. *In vitro* gaz production

In the process of ruminal cell wall digestion, high lignin content acts as a physical barrier due to its close association with polysaccharides. This structural complexity hinders microbial access to fermentable carbohydrates in the rumen, ultimately limiting digestibility (Machado et al., 2020).

Table 2 presents the gas production characteristics of the substrates. The untreated DP showed the highest value for the soluble fraction (a), which was significantly greater than all treated substrates ($P < 0.05$). This can be attributed to the higher content of soluble sugars in the untreated substrate, resulting in increased gas production from the rapidly fermentable fraction.

Gas production from the insoluble but degradable fraction (b) was significantly higher in all treated substrates ($P < 0.001$). This increase is likely due to the reduction and structural modification of polyphenols during treatment, which reduced their inhibitory effects and enhanced microbial activity. Phenolic compounds in ruminant feed are known to impair microbial growth, activity, and the production of volatile fatty acids in the rumen (Zimmer and Cordesse, 1996). Moreover, the treatments likely disrupted the chemical bonds between lignin and carbohydrates, thereby improving access for cellulolytic and hemicellulolytic microorganisms to the fibrous components of the cell wall (Morot-Gaudry, 2010).

The highest values for potential gas production (a + b) were recorded in DPUF and DPUL treatments ($P < 0.001$), reflecting the synergistic effect of urea and fungal fermentation in enhancing substrate biodegradability. The gas production rate (c) remained unchanged across all treatments.

Fig. 8 depicts the cumulative gas production measured over a 96-hour incubation period. The most substantial increase in gas production occurred within the first 48 hours, especially for the DPUF and DPUL treatments. These observations align with the data presented in Table 2, reinforcing the superior efficacy of the combined urea and solid-state fermentation (SSF) co-treatment.

The enhanced gas production observed in the urea/SSF co-treated substrates can be attributed to the swelling effect induced by urea in an aqueous environment, which facilitates greater accessibility of the plant material to both fungal mycelium and ruminal cellulolytic microorganisms (Chenost and Kayouli, 1997). Furthermore, nitrogen supplementation has been demonstrated to stimulate cellulolytic activity in rumen microbes (Chenost and Dulphy, 1987).

In summary, the synergistic interaction between urea treatment and white-rot fungal fermentation effectively preserves the structural integrity of fiber while improving carbohydrate availability to ruminal microorganisms.

Consequently, these treatments resulted in enhanced fermentation characteristics, reflected by increased gas production and digestibility, despite only modest alterations in the lignocellulosic matrix (Datsomor, 2022).

The results for OMD, ME and VFA following treatments are summarized in Table 3. OMD significantly increased in all treated substrates, with the most notable improvements observed in DPUF and DPUL ($P < 0.001$). This enhancement is attributed to the reduction of cell wall components and the concomitant increase in CP content after fermentation (Sharma and Arora, 2010). Chemical treatment facilitates the cleavage of ester bonds linking lignin, hemicellulose, and cellulose, thereby disrupting the lignocellulosic matrix (Chenost and Kayouli, 1997). Additionally, white-rot fungi represent the only fungal group capable of degrading lignin and its associated structures, converting them into water and carbon dioxide (Dashtban et al., 2010; Hou et al., 2020). Consequently, the synergistic action of basidio-ammonification results in elevated CP levels and substantial lignin removal, thereby enhancing substrate hydrolysis by ruminal microbiota.

The estimated ME was significantly higher in the treated substrates, particularly in DPUL ($P < 0.01$). These improvements reflect enhanced fermentation efficiency and greater energy release, both of which are critical for optimizing animal performance. Additionally, a significant increase in VFA concentrations was observed in the treated groups ($P < 0.01$). The variations in VFA levels among the treatment groups align with the elevated gas production, especially notable at the 24 hour of incubation (Wuanor and Ayoade, 2017). During the in vitro incubation of feedstuffs with buffered rumen fluid, VFA and gases are produced as a result of carbohydrate fermentation (Blümmel and Ørskov, 1993). Among the treatments, DPUL yielded the highest total VFA, likely due to improved microbial access to fermentable carbohydrates. According to Datsomor (2022), this enhanced energy release is attributed to the synergistic effect of urea and SSF using *L.tigrinus*, which facilitates more efficient degradation of the lignocellulosic matrix.

3. Conclusion

This study demonstrates the potential of converting solid waste into higher-quality animal feed through targeted biological and chemical treatments. The findings indicate that fungal inoculation and urea treatment are promising strategies for upgrading low-quality DP into more digestible and nutritious feed for ruminants.

Interestingly, the combined treatment was less effective in reducing cell wall components and phenolic content than fungal treatment alone. This could be attributed to nitrogen accumulation during chemical treatment, which may inhibit the synthesis of ligninolytic enzymes. The moderate structural modification achieved through these treatments appears to strike a beneficial balance, enhancing digestibility while preserving sufficient fiber integrity to maintain effective rumen function. Extensive fiber degradation may impair rumen health, but the partial breakdown observed in this study likely improved microbial accessibility without compromising fiber effectiveness.Moreover, the moderate reduction in phenolic content seems adequate to mitigate their inhibitory impact on rumen microbes while retaining some antioxidant potential.

Further in vivo studies are needed to confirm digestibility improvements and assess the nutritional efficacy of these treatments in livestock. Overall, these findings support the use of such techniques not only for enhancing feed quality but also as a sustainable approach to managing agricultural waste and addressing environmental concerns. In particular, such treatments represent a low-cost, low-energy bioprocess with potential for large-scale application, enabling the valorization of lignocellulosic by-products while reducing waste accumulation and enhancing feed availability in a practical and eco-friendly manner.

Abbreviations

SSF: solid state fermentation ; *DP*: untreated date pedicels; *DPU*: urea treated date pedicels; *DPF*: SSF treated date pedicels with *Fomes fomentarius*; *DPL*: SSF treated date pedicels with *Lentinus tigrinus*; *DPUF*: Urea/SSF co-treated date pedicels with *Fomes fomentarius*; *DPUL*: Urea/SSF co-treated date pedicels with *Lentinus tigrinus*; *NDF*: neutral detergent fiber; *ADF*: acid detergent fiber; *ADL*: acid detergent lignin; *CP*: crude protein; *OMD*: organic matter digestibility; *ME*: metabolizable energy; *VFA*: volatile fatty acid; *GP*: net gas production

CRediT authorship contribution statement

Olfa Abid: Conceptualization, Writing-original draft, writing-review and editing, format analyses and investigation. **Itaf Chebbi:** format analyses and investigation. **Taha Najar:** funding acquisition, Supervision. **Atef Jaouani:** Conceptualization, writing-review and editing, funding acquisition, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets and materials used during the current study are available from the corresponding author upon reasonable request.

Ethical approval

The article does not contain any studies with human participants. It also does not perform experiments directly on animals, so, these experiences not need ethics statement.

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Table 1

Effect of Urea treatment, SSF treatment and SSF/Urea co-treatment on the cell wall component value (% DM basis) of date pedicels.

Item	NDF	ADF	ADL	Celluloses	Hemicelluloses
DP	68,72^{a}±0,46	44,59^{a}±1,11	20,04^{a}±1,12	24,55^{a}±2,23	24,13^{abc} ±0,65
DPU	64,98^{b}±0,49	41,68^{b}±1,35	17,83^{bc}±0,70	23,30^{a}±1,84	23,30 ^{bc}±1,84
DPF	59,71^{d}±0,99	33,18^{c}±0,62	18,99^{ab}±0,55	14,24^{b}±1,17	26,53^{a} ±1,61
DPL	53,81^{e}±0,51	31,44^{c}±1,56	16,99^{c}±0,47	14,45^{b}±2,03	22,37^{c}±1,05
DPUF	64,99^{b}±0,49	38,02^{d}±0,77	18,95^{bc}±0,54	19,57^{c}±2,09	26,97 ^{a}±1,33
DPUL	63,08^{c}±1,06	37,18^{d}±0,84	17,38^{ab}±0,09	19,80^{c}±0,87	25,90^{ab}±0,28
P-value (Treatments effects)	***	***	*	**	*

Values are means ± standard error. Means within a column with different superscripts (a, b, c…) differ significantly at P < 0.05 (Duncan test).

****P*-value < 0.001; ***P*-value < 0.01;**P*-value < 0.05; NS not significant; DP, untreated date pedicels.

NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber; ADL = Acid Detergent Lignin.

DPU, urea treated date pedicels; DPF, SSF treated date pedicels with *F.fomentarius*; DPL, SSF treated date pedicels with *L.tigrinus*; DPUF, Urea/SSF co-treated date pedicels with *F.fomentarius*; DPUL, Urea/SSF co-treated date pedicels with *L.tigrinus*

Urea was applied at 4% (w/w) and incubated at 30 °C for 8 days; SSF was performed at 28 °C for 22 days.

Table 2

Gas production kinetics of date pedicels following Urea treatment, SSF treatment and SSF/Urea co-treatment

Item	Kinetic of gas production			
	a (ml)	b (ml)	c (ml/h)	a+b (ml)
DP	-0,91[a]	12,44[a]	0,035[a]	11,53[a]
DPU	-1,82[b]	15,89[b]	0,055[a]	14,06[b]
DPF	-2,39[b]	18,16[c]	0,058[a]	15,77[bc]
DPL	-2,17[b]	18,67[c]	0,053[a]	16,49[c]
DPUF	-2,2[b]	19,29[c]	0,055[a]	17,08[c]
DPUL	-2,38[b]	20,35[c]	0,061[a]	17,96[c]
SEM	±0.17	±0.81	±0.003	±0.66
P-value (Treatments effects)	*	***	NS	***

[a,b,c. . .] Means in the same column with different super scripts are significantly different at $P < 0.05$ (Duncan test).

All values are mean ± standard error; ****P*-value < 0.001; ***P*-value < 0.01;**P*-value < 0.05; NS not significant.

SEM, standard error of the mean.

a (ml), gas production from the soluble fraction;

b (ml), gas production from insoluble but degradable fraction;

c (ml/h),gas production rate;

a+b (ml), potential gas production.

DP, untreated date pedicels; DPU, urea treated date pedicels; DPF, SSF treated date pedicels with *F.fomentarius*; DPL, SSF treated date pedicels with *L.tigrinus*; DPUF, Urea/SSF co-treated date pedicels with *F.fomentarius*; DPUL, Urea/SSF co-treated date pedicels with *L.tigrinus*

Urea was applied at 4% (w/w) and incubated at 30 °C for 8 days; SSF was performed at 28 °C for 22 days.

Table 3

In vitro fermentation parameters (OMD, ME, VFA) of date pedicels treated with Urea treatment, SSF treatment and SSF/Urea co-treatment

Item	OMD (%)	ME (MJ/kg DM)	VFA (mmol/300 mg DM)
DP	21,89 [a]	3.06 [a]	0.14 [a]
DPU	27,38 [b]	3.58 [b]	0.22 [b]
DPF	26,72 [b]	3.76 [ab]	0.25 [ab]
DPL	26,63 [b]	3.76 [ab]	0.25 [ab]
DPUF	29,53 [c]	3.87 [ab]	0.26 [ab]
DPUL	30,81 [c]	4.06 [c]	0.29 [c]
SEM	0,861	0.098	0.015
P-value (Treatments effects)	***	**	**

[a,b,c. . .] Means in the same column with different super scripts are significantly different at $P < 0.05$ (Duncan test).

All values are mean ± standard error; ****P*-value < 0.001; ***P*-value < 0.01;**P*-value < 0.05; NS not significant.

SEM, standard error of the mean.

OMD, organic matter digestibility (%); ME, metabolizable energy (MJ/kg DM); VFA, volatile fatty acid (mmol/300 mg DM).

DP, untreated date pedicels; DPU, urea treated date pedicels; DPF, SSF treated date pedicels with *F.fomentarius*; DPL, SSF treated date pedicels with *L.tigrinus*; DPUF, Urea/SSF co-treated date pedicels with *F.fomentarius*; DPUL, Urea/SSF co-treated date pedicels with *L.tigrinus*

Urea was applied at 4% (w/w) and incubated at 30 °C for 8 days; SSF was performed at 28 °C for 22 days.

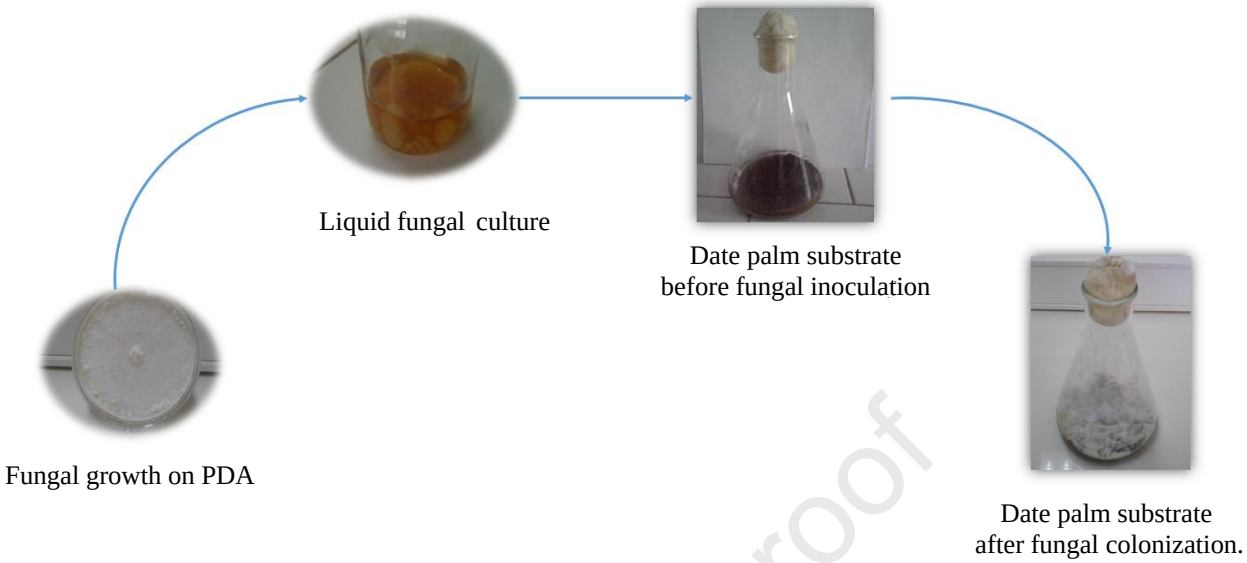

Fig. 1. Steps of fungal pretreatment of DP

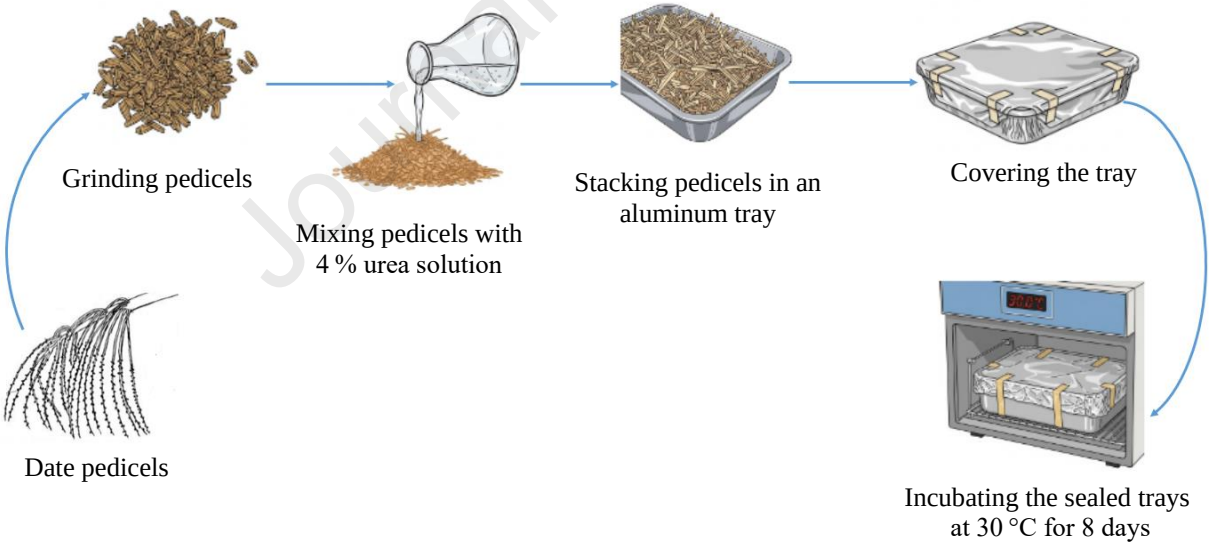

Fig. 2. Urea Treatment Process of DP

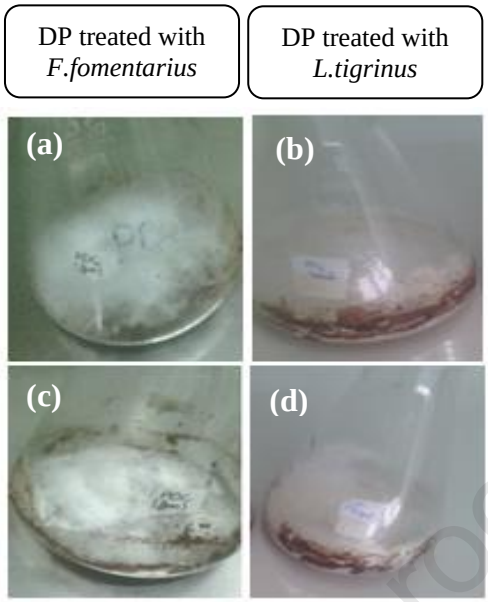

Fig. 3. Mycelial growth on DP after 22 days of SSF treatment (a,b) and SSF/Urea co-treatment (c,d)

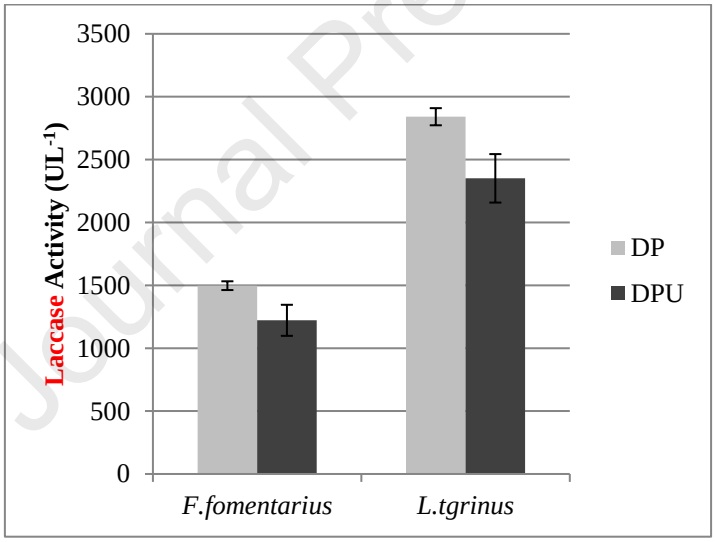

Fig. 4. Laccase production in solid-state cultures of *F. fomentarius* and *L.tigrinus* cultivated on DP and DPU. DP, untreated date pedicels; DPU, date pedicels treated with 4% urea (w/w), 30 °C, 8 days. SSF was performed at 28 °C for 22 days.

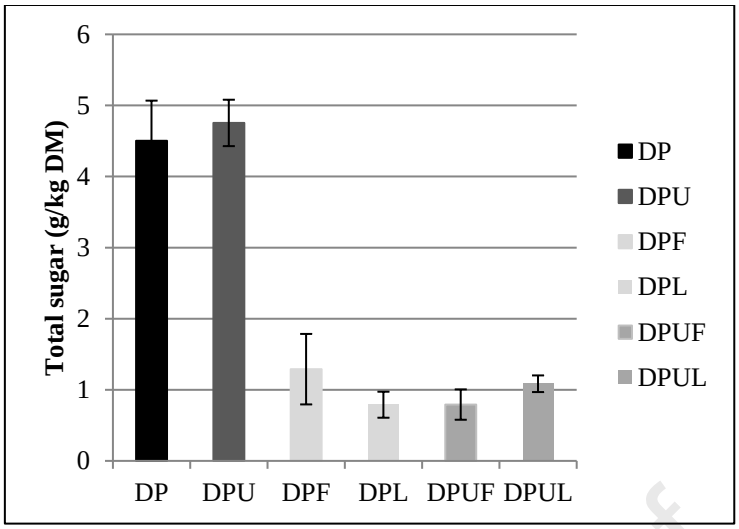

Fig. 5. Effect of Urea treatment, SSF treatment and SSF/Urea co-treatment on total sugar content.
DP, untreated date pedicels; DPU, urea treated date pedicels treated with 4% urea (w/w); DPF, SSF treated date pedicels with *F.fomentarius* (28 °C, 22 days); DPL, SSF treated date pedicels with *L.tigrinus* (28 °C, 22 days); DPUF, Urea/SSF co-treated date pedicels with *F.fomentarius*; DPUL, Urea/SSF co-treated date pedicels with *L.tigrinus*

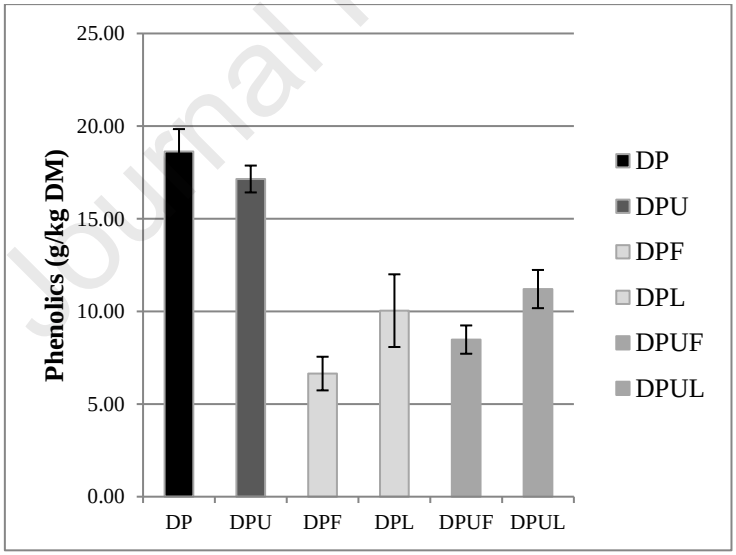

Fig. 6. Effect of Urea treatment, SSF treatment and SSF/Urea co-treatment on phenolics content.
DP, untreated date pedicels; DPU, urea treated date pedicels treated with 4% urea (w/w); DPF, SSF treated date pedicels with *F.fomentarius* (28 °C, 22 days); DPL, SSF treated date pedicels with *L.tigrinus* (28 °C, 22 days); DPUF, Urea/SSF co-treated date pedicels with *F.fomentarius*; DPUL, Urea/SSF co-treated date pedicels with *L.tigrinus.*

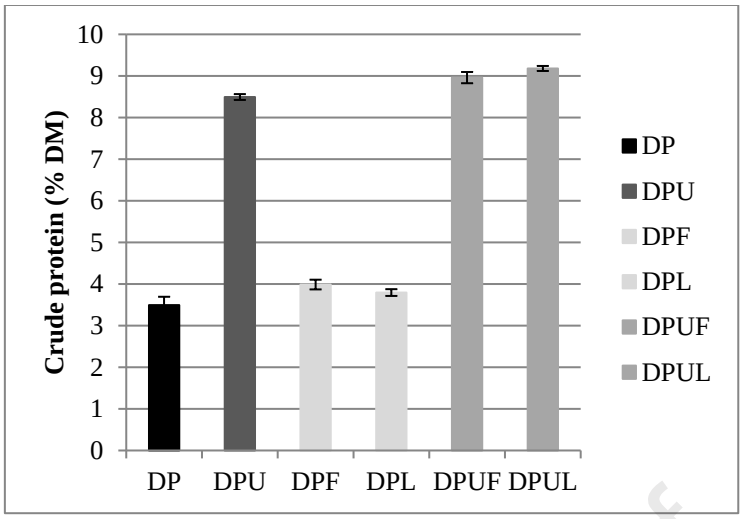

Fig. 7. Effect of Urea treatment, SSF treatment and SSF/Urea co-treatment on CP content.

DP, untreated date pedicels; DPU, urea treated date pedicels treated with 4% urea (w/w); DPF, SSF treated date pedicels with *F.fomentarius* (28 °C, 22 days); DPL, SSF treated date pedicels with *L.tigrinus* (28 °C, 22 days); DPUF, Urea/SSF co-treated date pedicels with *F.fomentarius*; DPUL, Urea/SSF co-treated date pedicels with *L.tigrinus*

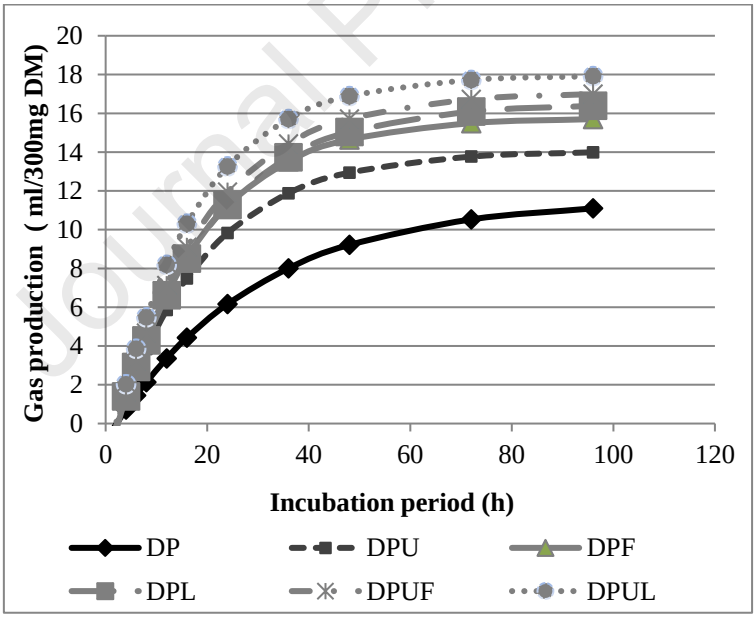

Fig. 8. Effect of Urea treatment, SSF treatment and SSF/Urea co-treatment on *in vitro* gas production.

DP, untreated date pedicels; DPU, urea treated date pedicels treated with 4% urea (w/w); DPF, SSF treated date pedicels with *F.fomentarius* (28 °C, 22 days); DPL, SSF treated date pedicels with *L.tigrinus* (28 °C, 22 days); DPUF, Urea/SSF co-treated date pedicels with *F.fomentarius*; DPUL, Urea/SSF co-treated date pedicels with *L.tigrinus.*

Highlights:

- Urea, fungal SSF, and their combination upgraded date pedicels for ruminant feed.
- Results showed that urea treatment increased crude protein content.
- SSF reduced significantly cell wall fiber fractions, improving digestibility.
- Combined urea/SSF showed highest gas production, indicating optimal rumen function.

Declaration of Interest Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.