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Effect of Soaking and Urea Treatment on the *In Sacco* Organic Matter Degradability of Whole Wheat Straw at Various Incubation Times in Fistulated Sheep

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Abstract

Six kg whole wheat straw (Claire variety) samples were taken randomly from a small bale and either kept dry (Soaking 0:1) or soaked (Soaking 2:1) in tap water (2 units' water: 1 of straw) and left for 16hs. Urea solutions were prepared according to the concentration of urea in straw by thoroughly mixing with 150 ml tap water in a garden hand sprayer. These solutions were mixed with the straw in plastic bags which were compressed to eliminate air, labelled, securely tied and stored in a room at 18˚C - 24˚C for 28 days. *In-sacco* studies were performed on two permanently fistulated sheep fed on a basal diet of 410 g concentrates and 820 g chopped hay/head/day over two time periods according to a 2 × 2 cross over design. Approximately 4 g of untreated and treated air-dried samples were weighed into nylon bags, which were placed in the rumen of these sheep for 0, 6, 18, 72, 48, 24, and 96 h. At the end of each incubation time, the bags were removed from the rumen and washed under running cold water until the rinsing water was colourless. Then the bags were oven-dried at 60˚C for 24 h and the un-degraded residues were weighed to estimate the dry matter degradability and the dried residues were ashed at 600˚C for 6 h to estimate the organic matter degradability of whole wheat straw. Only the OMD data were statistically analysed for this paper to compare the effect of soaking and urea treatments and their interaction on OMD at each incubation time at $P < 0.05$. Soaking showed a negative effect on the OMD of whole straw at most incubation hours, the urea treatment improved the OMD of straw at almost all incubation times. The OMD was significantly increased when urea was increased from 0 to either 2.5 or 5%. The final conclusion of the

current study showed that treating straw with urea at different levels 2.5 and 5% increased OMD at various incubation times. On the other hand, soaking with water significantly reduced the OMD at most incubation hours.

Subject Areas

Agricultural Science

Keywords

Whole Wheat Straw, Degradability, Fistulated Sheep, Incubation Hour

1. Introduction

Many researchers have tried to improve the nutritive value of straw by chemical and biological means [1] [2]. Some researchers have used the nylon bags [3]. Treatment with chemicals, such as urea alters the characteristics of straws and renders cell wall constituents more vulnerable to microbial attack in the rumen, resulting in higher digestibility and animal intakes by ruminant animals. Various authors [4]-[6] have confirmed that the degradation of urea into ammonia can be accelerated by increasing moisture content during straw treatments. The objective of this study was to investigate the effect of the application of soaking and urea levels [7] on the *in-sacco* organic matter degradability [8] [9] showed that in the previous study, the urea treatment at either 2.5 or 5% improved the *in sacco* OMD when compared with the control [10] at all incubation hours [11]. However, the higher soaking ratio showed lower OMD than the low soaking ratio at most incubation hours [12]. The aim of the study was to investigate the effect of soaking and urea treatment on the *in-sacco* organic matter degradability of whole wheat straw at various incubation times in fistulated sheep.

2. Material and Methods

2.1. Experimental Design

The straw treatments were performed as a factorial experiment by involving a 1 × 2 × 3 design in duplicate to study the effect of soaking ratios and urea levels on the chemical composition of the whole wheat straw as the following: whole wheat straw, two soaking levels 0:1 [13] and 2:1 (two parts of water and one part of straw) and three levels of feed grade urea (0, 2.5 and 5% w/w). While the degradability trial was designed according to a split-unit design involving two fistulated sheep (blocks), seven incubation times (0, 6, 18, 24, 48, 72 and 96 h), one tested straw (whole wheat straw), six straw treatments and two periods to study *in sacco* degradability of treated and untreated straws in duplicate. Only 8 sample bags could be inserted within the rumen of each of the two sheep at any incubation period due to the legal restrictions imposed by the Home Office. Moreover, there were only 2 fistulated sheep available in the research facility at

Cockle Park. Therefore, it was not possible to insert more than 3 pairs of bags from the same soaking ratio with three different levels of urea in each sheep at each incubation time and each period. However, due to the limited rumen space in fistulated sheep, the treated straws were grouped into 2 groups of 3 treated straws in duplicate per group as shown in Table 1. These straw groups were then tested for their *in sacco* OM degradability for each incubation time by using 2 fistulated sheep over two periods.

Group A = soaking 0:1, where no water and one part of wheat straw at the three urea levels (0, 2.5 and 5%). Group B = soaking 2:1, where two parts of water and one part of wheat straw at the three urea levels (0, 2.5 and 5%).

Table 1. Allocation of treated and untreated straws at different periods to 2 sheep.

Periods	Sheep 1	Sheep 2
1	A	B
2	B	A

The duration of the trial during the first and second periods was 58 days. Each period of the trial involved 7 - 9 days of adaptation of sheep to individual pens 11 days of insertion and removal of bags and 10 days of group housing.

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2.2. Collection and Preparing of Straw

Six kg of whole wheat straw (Claire variety) was taken randomly from a small bale and kept intact. Then the dry matter content of whole wheat straw was determined. Each kg of straw was packed in black plastic bags of size 10 × 29 × 38 inches to be ready for application of water and urea according to the experimental design as described below.

2.3. Application of Water

The whole straw was soaked separately with tap water by a garden hand sprayer. The water was sprayed on the straw inside the bags to avoid any losses of water during the spraying process and then the bags were closed and tied to prevent any losses of water by drainage or evaporation then the bags were left for 16h to allow the water to penetrate most straw fibres inside the bags in order to maximize the effect of soaking.

2.4. Application of Urea

The urea utilized in this trial was urea of animal feed grade, which contained 42% nitrogen and was supplied by *Chance and Hunt Limited Alexander House, Crown Gate, Runcorn, Cheshire WA72UP*. Each concentration of the urea solutions was prepared by mixing 0 g urea/kg of wheat straw, 25 g urea/kg whole wheat straw and 50 g urea/kg of whole wheat straw. Each urea level was thor-

oughly mixed with 150 ml of tap water in a compressed air, garden hand sprayer. The resultant urea solution was sprayed on the whole wheat straw inside the bags and mixed by hand for 10 minutes to ensure thorough mixing of urea solution with the whole wheat straw. When the mixing was completed, each bag was compressed to eliminate air, securely tied, labelled and stored at room temperature of around 18 - 24˚C for four weeks.

2.5. Sheep and Housing

The trials were performed on two mature sheep of the Walsh Llyen breed at average weights of 82 kg, ±1.0 SEM and about 9 years old. Each whether fitted with a permanent rumen fistula (2.5 cm diameter) was used to estimate *in sacco* OMD of untreated and treated wheat straws. The two fistulated sheep were weighed three times during each individual period. They were weighed, prior to their transfer into individual pens and reweighed again twice at the beginning of the insertion of bags. Eventually, on the last day of the trial, they were moved into group housing.

2.6. Feeding Routines

The requirements of fistulated sheep of this study were calculated according to the equation proposed by [14]. The calculated daily requirements of the sheep were 1250 g/head/day with about 2:1 ratio of chopped hay and concentrate diet. The ingredient composition of concentrate and the chemical composition of the basal diets offered to the fistulated sheep shown are in **Table 2** and **Table 3** respectively. During the adaptation period, the basal diet was monitored and some alterations were made due to the large refusal of hay, and hence, the quantity of grass hay was reduced to 820 g and compensated by a slight increase of concentrate to 410 g. Thus, the two fistulated sheep received a daily basal diet comprising of 820 g/head/day of chopped hay and 410 g/head/day of concentrate. The daily feed was offered in 2 equal portions at 9.30 am and 16.30 pm where these sheep had free access to fresh and clean water in separate buckets. The concentrate allowance was always offered first to the sheep so that it was consumed within 10 - 15 minutes before chopped hay was offered to these sheep.

Table 2. Ingredient composition of concentrate used to feed the fistulated sheep.

Composition of concentrate	g/kg
Soybean meal	200
Maize gluten feed	150
Rolled barley	275
Sugar beet pulp	250
Soy pass	25
Molasses	75
Vitamin and mineral supplements	25
Total	1000

Table 3. The means for the nutrient compositions (g/kg DM) of the basal diets offered to the fistulated sheep during the first and second period of the *in-sacco* trial.

Composition	Types of feed	
	Concentrate	Chopped hay
Dry matter (g/kg)	862.3	897.9
Organic matter	918.7	931.4
Crude protein	167.3	122.9
Ether extracts	21.3	11.2
Neutral detergent fibre	169.8	528.3
Acid detergent fibre	66.7	294.0
Acid detergent lignin	12.6	36.8

2.7. Numbering of Polyester Bags and Incubation of Straws

Around 10 × 20 cm polyester bags (53 μm pore size) purchased from Bar Diamond Inc, USA, were employed for the incubation of whole wheat straw. The bags were clearly labelled using permanent marker (water-resistant marker). The OM degradability of the differently treated straws was measured by *in-sacco* incubation of 4g air-dried samples, in the rumens of the two permanently fistulated sheep and removing them at 0, 6, 18, 24, 48, 72, and 96 hours of incubation. The arrangements of the experiments were performed on the whole straw over two periods of the two fistulated animals. The procedure of incubation was carried out according to [3]. The bags were checked for any holes or rips, labelled, weighed empty and filled with about 4g dried sample and tied separately with 65 cm long metal chain weighing about 55 g. Each chain carried three pairs of bags (three duplicates), where each bag was secured with a strong string and clinched into a link of the chain at the 6^{th} chain link intervals, between each bag to prevent overlapping of bags inside the rumen to facilitate the withdrawal of bags from the rumen via the fistula. The other end of the chain was tied to a metal ring linked with the stopper on the fistula, where distance between the top of the cannula and the last bag was about 15 cm. The bags were placed in the rumen of two fistulated sheep, before the morning meal and incubated for different incubation times (0, 6, 18, 72, 48, 24, and 96h). At the end of each incubation interval, the bags were removed from the rumen and thoroughly washed under a stream of running cold tap water for almost 15 minutes until the rinsing water was colorless. Then the bags were dried in the oven to constant weight at 60˚C for 24 h. The proportion of DM, that had disappeared, was calculated from the amount that was left in the bags after each incubation and the dried residues were ashed at 600 C for 6h to estimate OMD for each incubation time.

3. Chemical Analyses

Samples of treated straw before and after *in-sacco* incubation were collected and oven-dried at 60˚C for a period of 24 hours and then passed through a 2 mm

sieve laboratory hammer mill for their chemical analyses. The basal diet (concentrates plus chopped hay) and the whole wheat straw were analysed in duplicate for DM, OM, CP and EE according to the standard procedures of [15]. Acid detergent fibre, neutral detergent fibre and acid detergent lignin were analysed as described by [16] [17] and later modified by [18]. Omitting sodium sulphate and α amylase and expressed inclusive of residual ash only for NDF analysis. Hemicellulose and cellulose were calculated from the data of NDF and ADF as suggested by [19]. Crude protein (CP) was determined by using LECO FP 428 Nitrogen determinator and the nitrogen content was converted to crude protein by multiplying N by 6.25.

4. Calculations

The Organic Matter Degradability (OMD) of Whole Wheat Straw

DM or OM Disappearance g/kg =

$$\frac{\text{Initial DM or OM (g)} - \text{Final DM or OM (g)} \times 1000}{\text{Initial DM or OM (g)}}$$

Statistical Analysis

The rate and extent of *in-sacco* degradability of whole wheat straw data were performed by using the spreadsheet (Microsoft Excel) and statistically analysed by using the Minitab software package respectively. The model studied the main effect of soaking, urea, sheep and period and OM at various times (Cross over factorial arrangement).

5. Results

The means for the main effects of soaking and urea on OMD are presented in Figures 1-2 respectively. While soaking showed a negative effect on the OMD of whole straw at most incubation hours ($P < 0.01$). While the urea treatment improved OMD of straw at almost all incubation times. The OM disappearance was significantly increased at 0, 24, 48, 72 and 96h of rumen incubation when urea was increased from 0 or 2.5 to 5% ($P < 0.01$, $P < 0.001$ and $P < 0.05$) respectively.

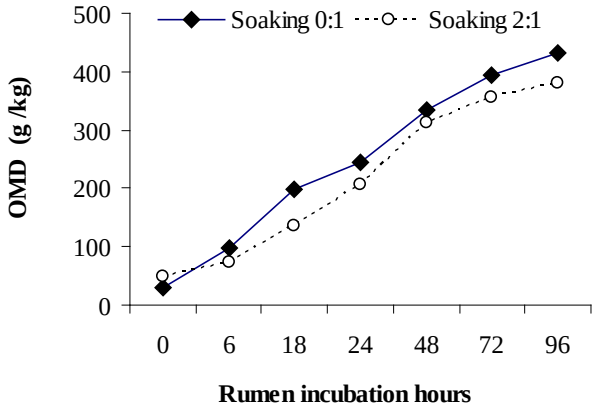

Figure 1. Main effect of soaking on OMD.

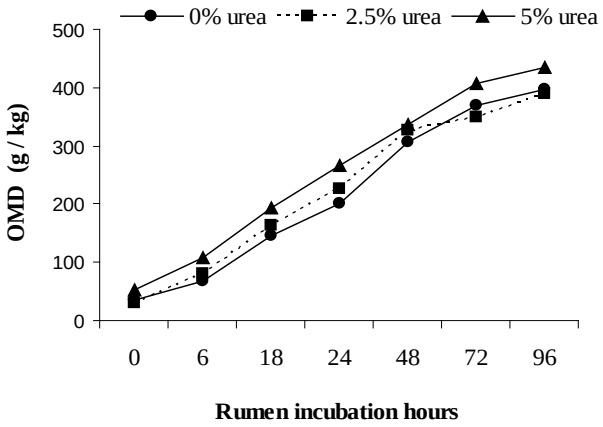

Figure 2. Main effect of urea levels on OMD.

Whereas, at 6 and 18h of rumen incubation the OM disappearance was significantly increased when urea was either increased from 0 to 2.5 or 5% ($P < 0.001$). The OM disappearance increased as the time of incubation increased. Moreover, the OM disappearance increased as rumen incubation hours increased whether samples had been soaked or not. At time 0, soaking significantly increased but at all other hours, soaking reduced OM disappearance ($P < 0.05$).

6. Discussion

6.1. Effect of Soaking Ratio on OM Degradability of Whole Wheat Straw

Soaking reduced the OMD of whole wheat straw throughout the incubation hours except at 24 and 48 h where they were increased, which was in the same line proved by [9] in the previous study that showed the higher soaking ratio lower OMD than the low soaking ratio at most incubation hours ($P < 0.01$) the negative effect of soaking could partly be attributed to the excess use of water, which was observed to be present at the bottom of these bags even after 28 days of storage. This excess water may have washed some of the solubles from the straw and so may have been the reason for the lower OMD with soaking at various times or could be attributed to the presence of high amount of water, which may have removed most of the soluble nutrients from the treated straw. In the absences of these soluble nutrients the rumen microbes were perhaps unable to degrade sufficient straw or possibly lignin infiltrates into the pore-spaces, previously occupied by water, between the molecules of non-cellulosic polysaccharides in the middle lamella and primary and secondary walls. The infiltrated hydrophobic lignin overlying these polysaccharides is an effective barrier to the enzymes that hydrolyse polysaccharides [20]. If lignin is also covalently bridged to cell wall polysaccharides, then the interfacial association between the two will make a complex resistant to the swelling action of aqueous solvents and, therefore, to microbial degradation. This may be due to the variation of phenolic compounds such as p-coumaric (PCA) and ferulic acids (FAs). An increase in the PCA concentration is associated with higher NDF, acid detergent fibre

(ADF) and lignin contents and lower IVDMD and crude protein content [21]. Or may be due to cross-link lignin to the structural carbohydrates of plant cell walls [22]-[24], through either ester-or ether-linkages [25]. *In vitro* esterification of forage cell walls with PCA or FA results in decreased digestibility of structural carbohydrates [26]. The effect of moisture level on the digestibility of urea treated straw (UTS) has also been variable. [27] reported a significant increase in straw degradability by lowering the straw DM from 750 to 450 g/kg, whereas [28] found that DM digestibility with the least water (0.2 litres: 1 kg straw) was highest, and that digestibility tended to decrease with increasing amounts of moisture in urea treated straw. The high contents of indigestible plant materials, lignin and silica, in ADF probably accounted for its low digestibility. Similarly, it has been found that urea treatment of rice straw [29] [30] or 4%, in straw treated with urea but without additional water, to 46.9%, in straw treated with urea and 250 ml of water/kg, to 53.1%, in straw treated with urea plus 337 ml of water/kg barley straw [31] significantly improved *in vitro* OMD.

This effect is likely to be associated with the higher bacterial and protozoal mass in the rumen with the urea treated straw-based diets. These large variations in degradability may be ascribed to the cell walls containing most of the plant components resistant to digestion in the rumen by the enzymes of micro-organisms. With advancing crop maturity, leaf-to-stem ratios decrease and changes occur in the structure and composition of cell walls as reported by [32] who revealed that in whole wheat straw plant during maturation; the feed nutritive value of leaf blades (LB) is highest, then leaf sheath, and that of the stem the lowest. The increases in OMD may be attributed to the decrystallization of the lignocellulosic complex and perhaps the breakage of other cell wall structures during grinding. Such increases were presumably the result of a more exposed surface area of the particles for microbial attack during the incubation with the rumen fluid. Furthermore, soaking was effective perhaps in converting insoluble components into soluble substances presumably through hydrolysis of the bonds, between the various cell wall components [33] [34]. This can be attributed to the moisture content of the whole straw and their fractions. Such a conclusion is in agreement with the improvement of the nutritional value of rice straw (RS) due to its high moisture content. Nevertheless, a very high content of moisture may not be encouraged, as [35] observed that the higher the initial moisture content of sugarcane bagasse, the greater the steam consumption was needed for effective pre-treatment or may be attributed to the longer time straw samples remained in the bags with the rumen liquor, which may have caused the micro-organisms to enhance degradation. It could also be due to the distribution of insoluble ash and silica [36] or may be due to sample size, where reduction of particle size (2.5 g) could play a crucial role by increasing the effective surface area for the microbial degradation, which strongly agreed with the finding by [37] and [36] who reported that the damaged or cut cell wall surfaces are the primary sites for bacterial and protozoal action. [38] and [39] showed consistently in their results the effect of particle size reduction was more pronounced

at short incubation, which may be due to the difference in straw varieties [40] or animal species or rumen condition and the diet composition of the host animal [41]. An inverse relationship, between sample coarseness and dry matter disappearance was revealed by [42]. Likewise, the significant amounts of soluble phenolic-like compounds, especially trans P-coumaric acid released during soaking, which is toxic to rumen microbes and proved to be inhibitors to the rumen microbial digestion of plant cell walls *in-vitro* [43].

In addition, some phenolic acids are even toxic to micro-organisms by curbing their activities to degrade more lignin [44]. The lower degradability may also be attributed to the complicated botanical structures of nodes and internodes, which became more resistant for microbial enzymatic actions and for the absorption of water to increase the surface area of the particles which was essential to enhance further microbial degradation of different straw components. The effect of moisture level on the digestibility of urea treated straw (UTS) has also been variable. [27] reported a significant increase in straw degradability by lowering the straw DM from 750 to 450 g/kg, whereas [28] found that DM digestibility with the least water (0.2 litres:1 kg straw) was the highest, and that digestibility tended to decrease with increasing amounts of moisture in urea treatments probably accounted for its low digestibility. Similarly, it has been found that urea treatment of rice straw [29] [30] or barley straw [31] significantly improved *in-vitro* OMD. This effect is likely to be associated with the higher bacterial and protozoal mass in the rumen with the urea treated straw based diets. The effect of moisture on the degradability of wheat straw has been studied by [45]. These authors found that after 48 h degradability rose from 40.4%, in straw treated with urea but without additional water, to 46.9%, in straw treated with urea and 250 ml of water/kg, to 53.1%, in straw treated with urea plus 337 ml of water/kg. Despite the fact that all the treated straw was subjected to the same grinding process and therefore, should have had the same particle size (2b mm). They were also washed for the same time (30 mins/6 bags) yet it appeared that washing losses were still variable among the treated straws. This could probably be due to the proportion of the fine particles that can pass through the pores of the bags (size 53 μm) as proved earlier by [46]. The inefficient washing procedure post incubation could lead to an underestimation and possibly negative degradability values as reported by [47]. The loss of hemicellulose through its possible conversion to soluble oligo-and polysaccharides and their removal during washing might also have caused an increase in the degradability of straw as reported by [48]. Whereas, neutral detergent fibre bound N (NDF-N) significantly limited ($P < 0.05$) the rate and effective degradability of DM [3].

6.2. Effect of Urea Treatment on OM Degradability of Whole Wheat Straw

Since the rumen is the primary site for fibre degradation, the increases in degradability of the urea treated straws were presumably due to the increased susceptibility of structural carbohydrates of straws to rumen fermentation as well as

more energy being made available for the better growth of rumen microbes. These microbes tend to degrade straw as confirmed by [49] and [50]. The present findings showed that urea at 5 % was more affective in increasing OM degradability. In general, the higher the level of urea applied in the present study, the more degradable the treated straw became, which is in agreement with the previous finding by [9] that showed the urea treatment at either 2.5 or 5% improved *in-sacco* OMD as compared with the control (0% urea) at all incubation hours ($P < 0.001$). Moreover, [51] proved that the urea treatment was very effective in improving the degradability of sorghum stover and also they recommended the treatment of sorghum stover with urea for dry season feeding of cattle. The ureolysis process is affected by the doses of urea, moisture, and temperature. However, in this study, the straws treated with urea were stored at an uncontrolled room temperature where the temperature fluctuated from 10 - 20˚C. Moreover, urea at 2.5% with a higher level of moisture might have reduced the activity of urease enzyme, which led to the lower ammoniation and reduced concentration of ammonia which in turn reduced their effect on treated straws. This is in agreement with [52] who showed that urea treatment at high temperatures has increased urease activity; the optimum temperature for this enzymic activity in the soil is 30˚C, while it decreases as the temperature drops. However, urease activity can decrease as a result of both low and high temperatures. [53] observed lower urease activity with urea treatments at temperatures of 4˚C, and 35˚C. Likewise, [54] tested ammoniation at three levels of moisture (120, 300 and 450 g/kg straw), and reported that the highest DM digestibility was obtained at 300 g moisture/kg. The increased degradability may be due to reductions in NDF and ADF contents, which were due to the increased amounts of urea used to treat the straw. Both urea and NH_3 have the ability to dissolve parts of hemicellulose through the cleavage of ester bonds of uronic acids and removal of acetyl groups, and thus causing decreases in NDF and ADF [55] [56]. The partial damage caused by the urea treatment to the lignin-polysaccharide bond and the result of solubilisation of hemicellulose and lignin in straw, can expose the cellulose to microbial attack which consequently can increase the degradability of urea treated straws as revealed by [57] and [58]. The increased degradability can also be attributed to the reduction in crystallinity of cellulose and so the increased fragility of the cell wall, which consequently increases its susceptibility to attack by cellulolytic microbes. In a similar manner many other authors have listed the effectiveness of using urea in improving the nutritive vales of low-quality forages and have shown the following benefits; the chemical treatments enhance the nutritive value through increasing the number of accessible sites for the microbial attachment on the surface of the particles, increasing fibrolytic microbe quantity and hence fibrolytic enzyme activities, and improving rumen fermentation characteristics [59].

Treatment with chemicals, such as alkalis, alters the characteristics of straws and renders cell wall constituents more vulnerable to microbial attack in the rumen, resulting in higher digestibility and animal intakes [60]. The collapse of

vascular bundle sheath cells in treated rice straw [61], rupture of inner cuticular surfaces and separation from adjacent ground parenchyma when wheat straw was ammoniated [62], alteration in the friability of the rigid layer covering the inner surface of cell walls [57] and the ability of NH_3 to form an ammonia-cellulose complex and to decrease cellulose crystallinity can facilitate the increase in degradability of urea treated straws [63]. [64] indicated that under ordinary feeding conditions feed particles-associated bacteria are numerically predominant and occupy up to 70 - 80% of the total microbial population in the entire rumen contents in cows. [65] reported that the rate of fibre degradation depended on the extent to, which the rumen environment allows an adherent cellulolytic microbial population to develop. It could be possible that the urea treatment was able to increase the number of accessible sites for the microbial attachment on the surface of the straw due to their softness after the application of urea treatments in this study, which was supported by [66] who reported that the loss of small particles through the bag pores may have increased the value of soluble fractions in maize straw in comparison to ensiled apple pomace. Despite the fact that all the treated straw was subjected to the same grinding process and therefore, should have had the same particle size (2 mm). They were also washed for the same time (30 mins/6 bags) yet it appeared that washing losses were still variable among the treated straw. This could probably be due to the proportion of the fine particles that can pass through the pores of the bags (size 53 μm) as proved earlier by [46].

7. Conclusion

Treating straw with urea at different levels 2.5 and 5% increased OMD at various incubation times. On the other hand, soaking with water significantly reduced the OMD at most incubation hours.

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Conflicts of Interest

The authors declare no conflicts of interest.

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