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Monitoring of the evolution of the microbiological flora 'treated straw'

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ABSTRACT

The use of urea for the treatment of the straw constitutes an interesting alternative, particularly in developing countries. Its effectiveness depends, on one hand, on the intensity of the ureolysis and, on the other hand, on the effects of the ammonia produced on the composition of the straw and on the existent microbiological flora. The aim of this work is to study the effect of ammonia coming from urealyticum activity on the evolution of FMAT, total and fecal coliforms, fungus, yeasts and lactic bacterias. And this through two essays (straw + urea alone (U) and straw + urea molasses). The essays were done in conditions of humidity varying between 60 % and 70 %, during 30 days and at a temperature of 20 and 22°C. Acquired results are confronted to a blank (straw + water). As a result, the medium value of the pH is 9.2 for all essays treated by urea alone (U). It is 8.5 for the essay with molasses. The pH of the witness was only 5.5. FMAT is abundant in the witness. It reaches high levels ($1.7.10^7$ ufc/g) against 6.10^2 ufc/g for the essay with molasses. The lactic microbes were present in the essay with molasses with an abundance of $2.1.10^1$ ufc/g. They were more abundant in the witness ($1.7.10^4$ ufc/g). The same thing was observed with yeasts. In fact, at the level of the mix straw + urea + molasses, the average of the population of yeast is $4.9.10^1$ ufc/g. In the witness it reaches 4.10^4 ufc/g. These results can be explained by the influence of the high pH values in the essays with urea. The pH acts on the bacterial metabolism in general and on the ureolytic activity (hydrolysis of urea) particularly. The pH and/or the high concentration of ammonia formed can explain the statements stopped at the level of the growth of the studied microbiological floras.

KEYWORDS

Straw;
Urea;
Molasses;
Yeast;
Lactic bacteria.

INTRODUCTION

The improvement of the nutritional quality of the straw is due to several chemical processes: treatment using urea, ammonium sulfate, and molasses and biologic: use of adapted or combined ferments.

The treatment of the straw by urea only or by urea and molasses is for us a mean that satisfy the level of life of the most part of small breeders of our region. Such a complementation can be a factor of nutritional enrich-

ment of the straw in nitrogen, in minerals and in vitamins. These elements are essential for the improvement of meat or milk productions, and for both in case of mixed breeding. Several factors intervene in the process of transformation during the fermentation. We quote the intrinsic evolutions of the physico-chemical and microbiological parameters.

At the level of the literature, the studies realized in most part of developing countries especially in Africa, has shown on experimental livestock and industrial livestock clear improvements in both types of production (meat and milk). This, due to the complementation of the straw by nitrogen sources. Will such results have the same impacts on the livestock of the region of Gharb in Morocco? This last one is considered as the heart of the agricultural economy of the country. It is often subjected to difficult climatic conditions. The drought for several years and massive floods during the last two years. Winter crops (bersims, green) are either flooded in water at the time of the floods or burned or without production at the time of the drought.

During this work, we tried to see the possibility of improvement of the nutritional quality of the straw. This one is often bought from other regions or stored by the breeder in his farm. The hygienic quality is considered in order to avoid any kind of bad effects on the health of the cattle. Indeed, a monitoring of the dangerous groups of microorganisms has to be done. Those are the FMAT, the coliforms, the molds. Associated with the hygiene of the finished product, we have to bring to light the effect of the urea on the flora of technological interest (lactic bacteria and yeasts).

The evolution of the dynamic of the microbial populations is followed during a period of 30 days. This, in the aim of determining the various possible interactions between the urea, the bacterial growth and/or their ureolytic potential.

MATERIAL AND METHOD

Hacking of the straw

A straw bale is the cubic elementary unit of the storage in the cowshed. Each of the units is untied then put in a crusher at 1500 tours per min. The obtained mix is composed of fiber pieces of about 5 to 10 mm. It is stored in plastic barrels for a later treatment.

Treatment conditions of the straw

The mix is treated by various urea concentrations. The one having a urea proportion of 5 % is added in another essay of molasses. The incorporations were realized on samples at a humidity rate from 60 % to 70 % and placed at room temperature. The duration of the treatment was fixed at 30 days.

Used treatments

Essay 1: The proportions of ureas added to the straw are: 5 %, 10 %, 15 % and 20 %.

Essay 2: On the straw at 5 % of urea we added various proportions of molasses: 5 %, 10 %, 15 % and 20 %.

Essay 3: Witness: only straw.

Physico-chemical and microbiological analysis

(A) Physico-chemical analysis

The physico-chemical analysis essentially concerned the determination of the parameters pH, humidity and dry matter.

(a) Determination of the pH

The pH is measured by means of an Orien Research type pH meter. The values of the measure are taken after calibration of the device. The used standards are the pH 7 and 4.

(b) Determination of the humidity

The humidity is determined by passing 5g of the sample to analyze in a steam room at 105°C during 6 hours. The weight difference between the raw material and after drying was the tool of calculating the humidity value. The expression of the equation is the following one:

$$\text{Humidity (\%)} = (m2-T)/(m1-T)\times 100$$

where, m1 = essay sample (g), m2 = mass after drying (g), T = mass of the box (g)

(B) Microbiological analysis

Microbiological analyses were focused on the flora of hygienic interest and the flora of biotechnological interest.

(1) Preparation of the dilutions

10g of every sample are taken and added to 90 ml of sterile physiological water. The sample is put in an

REVIEW

TABLE 1 : Mean values of pH and humidity obtained after analyzing the samples of straw subjected to two types of treatment (straw + urea, straw + urea + molasses). Essays realized at room temperature and at initial relative humidity of 60 to 70 %

Sample	Mean of pH	Mean of humidity (%)
Witness (T)	5.50	66.50
	Essay (U)	
5%	9.27	63.80
10%	9.30	62.20
15%	9.25	60.40
20%	9.07	64.40
	Essay (UM)	
5%(U) + 5% (M)	8.64	63.40
5%(U) + 10%(M)	8.58	63.04
5%(U) + 15%(M)	8.58	61.63
5%(U) + 20%(M)	8.53	63.80

erlenmeyer of 250 ml. So we obtain a dilution to 10-1. Other dilutions (10-1 to 10-7) are successively done by putting 0.5 ml of the solution which is diluted in 4.5 ml of sterile physiological water.

(2) Seeding

The sowings are realized in a solid milieu. 1ml of the suitable dilution is put in a Petri box. 20 ml of gelose milieu previously sterilized at 120°C are then poured aseptically after cooling at the temperature of 45°C. The box is then homogenized then incubated in a steam room with adequate temperature. That depends on the nature of the group of microorganism to be studied. Every seeding is realized in three copies. Only the boxes having a number of colonies included between 30 and 300 are retained for the counting.

Determination of the quantity of total aerobic mesophilic flora (FMAT)

This group informs about the global bacterial load of the analyzed product. The TSA milieu truly relates the individuals of the total population that are in the studied milieu. It just has to be incubated at 30°C during 48 hours.

Determination of the quantity of fecal and total coliforms

They are germs that are accostumed to the digestive tract of the human or the animals. We also consider indicators of the hygienic quality of the product to be analyzed. The used milieu is the DCL (Desoxycholate Lactose Agar). The counting is made after 48 hours of incubation at a temperature of 37°C for the total coliforms and at 44.5°C for the fecal coliforms.

Determination of the quantity of yeasts

These germs are known for their biotechnological utilities. The evaluation of their abundance in a biotope is made by culture in the PDA milieu. And this after incubation during 48 hours at a temperature of 30°C.

Determination of the quantity of molds

The evolution of molds is realized in the PDA milieu after incubation during 72 hours at a temperature of 30°C.

Determination of the quantity of lactic bacteria

We do the same for germs of biotechnological utility. The most known milieu for their counting is the MRS. The colonies corresponding to this group can be counted after 24 hours of incubation at a temperature of 37°C.

RESULTS AND DISCUSSIONS

Results of the physico-chemical analysis

The results obtained for every studied parameter are reported in the form of tables. They are then analyzed and interpreted.

pH

The values of pH found from the various samples of both essays (straw + urea, straw + urea + molasses) are represented at the level of the TABLE 1.

For each of the proportions prepared with urea only, it was noticed that the pH was not able to decrease lower than 9. The average inter-proportion (straw-urea) is 9.2. It can be explained by the degradation of the urea molecule and the liberation of ammonia. The case is not the same for the mixture straw + urea + molasses. The average inter-proportion is equal to 8.5. The light decrease is probably due to the addition of the molasses. The sugars present in this last one can be the cause. For both essays, we noticed that the effect of the urea is more important than the other affects i.e. the microbial flora and the incorporation of the molasses. The fixed deductions are removed from the results obtained in the witness essay. The pH at this level gave an

average of 5.5.

The studies led by Badurdeen et al.[10] on the complementation by blocks of the urea treated or not treated straw has caused just a light decrease of the pH (mean value of 6.9). By using blocks with various rates of urea, Soetanto and Dixon[14] noted that the pH is not affected. The same result was observed more recently by Moujahed et al.[13] in the case of the complementation of the Acacia cyanophylla by the multinutritional blocks.

Humidity

The values of the humidity are also shown in the TABLE 1. Indeed, although the initial humidity is artificially obtained, this one did not know a considerable variation. The calculated averages oscillate from 62.70 % to 62.90 % respectively for both essays (straw + urea, straw + urea + molasses). The obtained averages constitute a favorable factor for the spontaneous proliferation of the microbial flora and the transformation of the raw material. Such humidity in the other plant types facilitated the installation of the flora of technological interest. This one has positively orientated the fermentation towards the improvement of the nutritional quality of the final product.

TABLE 2 : Evolution of the abundance of the FMAT in the straw according to time and to the urea concentration. Essays realized in barrels of 30kg at room temperature

Sample	ufc/g T0	ufc/g T30 Days	Mean for fmat ufc/g
Witness (T)	$6.08.10^8$	$1.85.10^7$	$3.96.10^7$
Essay (U)			
5%	00	00	00
10%	00	00	00
15%	00	00	00
20%	00	00	00

TABLE 3 : Evolution of the abundance of the FMAT in the straw at 5 % of urea according to time and to the various concentrations of molasses. Essays realized in barrels of 30kg at room temperature

Sample	ufc/g T0	ufc/g T30 Days	Mean fmat ufc/g
Essay (UM)			
5%(U) + 5% (M)	$2.20.10^2$	00	$5.2.10^2$
5%(U) + 10%(M)	$2.84.10^3$	00	$8.3.10^2$
5%(U) + 15%(M)	$1.18.10^3$	00	$3.4.10^2$
5%(U) + 20%(M)	$1.16.10^3$	00	$4.2.10^2$

Microbiological results

The results of all the microbiological analysis are in TABLE represented below.

An elementary interpretation will be done to every target group.

FMAT: Total aerobic mesophilic flora

The results of the evolution of the abundance of the FMAT in the straw according to time and to the urea concentration are illustrated in the TABLE 2. At the level of the witness, the evolution is normal. The abundance obtained at the end of the cycle of fermentation (30 days) a value of 3, 96.10^7 ufc/g. However, it is not the same with the straw treated with urea only. Whether it belongs to T0 or to T30, the FMAT is exterminated. The responsible factor is probably the urea.

With the molasses, the FMAT has recovered during the first week of fermentation. It is then eliminated at roughly the 30th day of fermentation. It seems that the population appeared during the first week of fermentation is the one returned with the molasses. This last one was not able to resist to the inhibitive effect of the urea or to one of her toxic transformation products such as the ammonia formed in the milieu.

TABLE 4 : Evolution of the abundance of the lactic bacteria in the straw according to time and to the urea concentration. Essays realized in barrels of 30kg at room temperature

Sample	ufc/g T0	ufc/g T30 Days	Mean for lactic bacteria ufc/g
Witness (T)	4.10^3	4.10^4	$1,7.10^4$
Essay (U)			
5%	00	00	00
10%	00	00	00
15%	00	00	00
20%	00	00	00

TABLE 5 : Evolution of the abundance of the lactic bacteria in the straw at 5 % of urea according to time and to the various concentrations of molasses. Essays realized in barrels of 30kg at room temperature

Sample	ufc/g T0	ufc/g T30 Days	Mean for lactic bacteria ufc/g
Essay (UM)			
5%(U)+5% (M)	$3,6.10^1$	00	$2,1.10^1$
5%(U)+10%(M)	$3,3.10^1$	00	$1,8.10^1$
5%(U)+15%(M)	$3,2.10^1$	00	$2,2.10^1$
5%(U)+20%(M)	00	00	00

REVIEW

TABLE 6 : Evolution of the abundance of the yeasts in the straw according to time and to the urea concentration. Essays realized in barrels of 30kg at room temperature

Sample	ufc/g T0	ufc/g T30 Days	Mean for yeasts ufc/g
Witness (T)	$3,5.\ 10^2$	$5,3.\ 10^4$	$1,8.\ 10^4$
Essay (U)			
5%	00	00	00
10%	00	00	00
15%	00	00	00
20%	00	00	00

TABLE 7 : Evolution of the abundance of the yeasts in the straw at 5% urea according to time and to the different molasses concentrations concentration. Essays realized in barrels of 30kg at room temperature

Sample	ufc/g T0	ufc/g T30 Days	Mean for yeasts ufc/g
Essay (UM)			
5%(U) + 5% (M)	$3,5.10^1$	00	$2,6.10^1$
5%(U) + 10%(M)	$2,7.10^1$	00	$3,3.10^1$
5%(U) + 15%(M)	$3,7.10^1$	00	$2,7.10^1$
5%(U) + 20%(M)	$4,5.10^1$	1.10^1	$4,9.10^1$

TABLE 8 : Evolution of the abundance of the molds in the straw according to time and to the urea concentration. Essays realized in barrels of 30kg at room temperature

Sample	ufc/g T0	ufc/g T30 Days	Mean for mold ufc/g
Witness (T)	$6,4.10^3$	00	$1,3.10^3$
Essay (U)			
5%	4.10^2	00	$6,0.10^1$
10%	00	00	00
15%	00	00	00
20%	00	00	00

TABLE 9 : Evolution of the abundance of the molds in the straw at 5% of urea according to time and to the different molasses concentrations. Essays realized in barrels of 30kg at room temperature

Sample	ufc/g T0	ufc/g T30 Days	Mean for mold ufc/g
Essay (UM)			
5%(U) + 5% (M)	00	00	00
5%(U) + 10%(M)	00	00	00
5%(U) + 15%(M)	00	00	00
5%(U) + 20%(M)	00	00	00

Lactic bacteria

The results of the evolution of the abundance of the lactic bacteria in the straw according to time and to various concentrations are described in the TABLE 4. It gives the same conclusions as those previously noticed. The lactic bacteria are present at very important levels even after 30 days of fermentation. It seems that the conditions are not hostile for this group of bacterium. The abundance registered at the end of the cycle of fermentation is 1, 7.10^4 ufc/g. However, once the urea is added even at reduced doses (5%), the lactic bacteria do not manage to multiply. A total absence of their population is noticed even at T0. It shows the very toxic effect of the urea on all the studied groups.

For the straw with 5% of urea mixed with various concentrations of molasses, the lactic bacteria are only present during the first day. And even for a molasses' concentration of 20%, we planned a persistence of the lactic bacteria during the end of the fermentation cycle. However, it is not the case.

Yeasts

The results of the evolution of the abundance of yeasts in the straw according to time and to the various concentrations of the urea are recorded in the TABLE 6. This last one shows that yeasts evolve in a very normal way at the level of the witness. The abundance of yeasts arrives at 1, 8.104 ufc/g. This result is lacking at the level of the essays added by urea. The urea confirms again that it is toxic for the group of yeasts. Their presence does not even appear in the low doses of urea (5 % of urea).

The same reports as previously are stopped with the straw at 5 % of urea and various concentrations of molasses. Yeasts manage hardly to show themselves at the first day of fermentation. Later they are eliminated by the presence of the urea. However, for a dose of 20% of molasses, we saw that the population of yeasts persists till the end of the cycle of fermentation. The abundance after 30 days of fermentation is roughly 10 germs by gram.

Essays of adaptations are going to be made in this sense to be able to arrive at abundance as important. This result is very interesting because of the importance of yeasts in the improvement of the nutritional quality of food commodities and in the general regulation of the metabolism at the ruminants.

Molds

The evolution of the abundance of molds in the straw according to the various concentrations of urea is described at the level of the TABLE 9. We noticed a presence at the beginning of the fermentation of molds with an abundance of 6, 4.10^3 ufc/g. This abundance decreases at the end of fermentation.

In the presence of urea, we noticed the total absence of molds in any essays that have been carried out. Thus, in the presence of 5% urea and various concentrations of molasses, molds were not able to appear even at the beginning of the fermentation.

It emerges from all the results that the urea is an excellent exterminator product for all the studied microorganisms. Essays of adaptation seem to be necessary for a better transformation of the straw.

The increase of $N\text{-}NH_3$ is especially due to the strong solubility of the urea and to its high speed of degradation. Several researches confirm this observation. Habib et al.[11] noted that the concentration in N-NH3 after the complementation of the rice straw by various types of multinutritional blocks. The same trend was also found by Badurdeen et al.[10] for the complementation of the treated or not treated straw in the urea by multinutritional blocks. Finally, it is important to note that the increase of the concentration in $N\text{-}NH_3$ is even more important as the rate of urea in blocks is high[14-16].

More recent studies showed that the complementation of the Acacia cyanophylla by blocks caused an important increase of the concentration of $N\text{-}NH_3$[13].

GENERAL DISCUSSION

The straw is almost the main nutritional source for cattle for most part of the breeders of the region of Gharb. This last one is one of the biggest regions of Morocco. It is known that the straw is less rich nutritionally. So, we proposed its improvement through treatments with urea only and with urea and molasses.

The reports of every treatment are estimated by the reading of the physico-chemical and microbiological results. We deduct from the essays that for each of the proportions prepared with the urea only, it was noticed that the pH was not able to decrease lower than 9. It can be explained by the degradation of the molecule of urea and the liberation of ammonia. The case is not the same for the mixture straw + urea + molasses. The average inter-proportion is equal to 8.5. The light decrease is probably due to the addition of the molasses.

The humidity averages calculated oscillate between 62.70% and 62.90%. Such values constitute a favorable factor for the spontaneous proliferation of the microbial flora and the transformation of the straw. Such humidity in the other plant types facilitated the installation of flora of technological interest. This one drives positively the fermentation to the improvement of the nutritional quality of the final product.

With the molasses, the FMAT was found during the first week of fermentation. It is then eliminated around the 30^{th} day of fermentation. It seems that the population appeared during the first week of fermentation is the one brought with the molasses. This last one was not able to resist to the inhibitive effect of the urea or to one of her transformation toxic products like the ammonia released in the milieu.

The lactic bacteria are present at very important levels even after 30 days of fermentation. It seems that the conditions are not hostile for this group of bacterium. The abundance recorded at the end of the cycle of fermentation is 1, 7.10^4 ufc/g. However, once the urea is added at reduced doses (5%), the lactic bacteria do not manage to multiply.

Yeasts manage hardly to show themselves the first day of fermentation. Later they are eliminated by the presence of the urea. However, for a dose of 20% of molasses, it has been shown that the population of yeasts persists till the end of the cycle of fermentation. The abundance after 30 days of fermentation is about 10 germs by gram.

Essays of adaptations are going to be made in this direction to be able to arrive at abundance as important. This result is very interesting because of the importance of yeasts in the improvement of the nutritional quality of food commodities and in the general in the regulation of the metabolism of ruminants.

We noticed a presence at the beginning of the fermentation of molds with an abundance of 6, 4.10^3 ufc/g. This abundance decreases at the end of fermentation.

It emerges from all the results that the urea is an excellent exterminator product of all the studied microorganisms. Essays of adaptation seem necessary for a better transformation of the straw.

REVIEW

CONCLUSION

The region of Gharb is subjected to difficult climatic conditions. Sometimes it is the drought which wins. It is the same for heavy rains accompanied with big floods. The flood of 2010 is an example. Most part of forage crops (lucerne, bersim) are either under the water or the yield of its products is reduced by a bad handling of the flooded crops.

The straw is the alternative food of the cattle in these difficult periods. Considering its poverty in nutritional elements, it was necessary for us to carry on a study on the improvement of its nutritional value. So, essays of treatment were adopted. The first series of treatment consisted in preparing a mixture composed of straw with urea only. The second series contained the straw, the urea and the molasses.

The various concentrations of the urea applied in the processing procedure constitute a toxic factor for all the studied groups of bacteria. However, the ureolytic activity remains very important. The hydrolysis reaction of the molecule of urea provokes a considerable quantity of ammonia revealed by its pungent smell. And it is probably, this atmospherical ammonia that discriminated the growth of the studied microorganisms. The addition of the varying quantities of molasses was not able to solve the problem of this toxicity.

Reactions of adaptation are going to be started to continue this work. It is a question of involving the competent microorganisms known for their biotechnological interest and arriving at a final product with nutritional characteristics that would be appreciated.

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