



# Modification of microbiological quality and valorization of different ruminants species ruminal content in farm animals feeding

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## RESEARCH ARTICLE

### Abstract

This study is based on the microbiological analysis of a common slaughterhouse by-product which is the rumen content, before and after treatment with HCl 1N, targeting its valorization in domestic animal feeding. A total of 24 rumen content samples were collected in pairs (two series) immediately after slaughter from 12 ruminants (cattle, sheep and goats). The first series was not treated with HCl (N0 = 12), while the second series was treated with HCl 1N (N1 = 12) to adjust its pH to about 2. Then all samples (series 1 and 2) were subject to a group of microbiological analyzes targeting identification and enumeration of total aerobic mesophilic flora; fecal and total coliforms; *Clostridium perfringens*; *Staphylococcus aureus*; *Salmonella spp.* as well as yeasts and molds. The results revealed that after lowering the pH to about 2, the 2<sup>nd</sup> series samples were considered of satisfactory quality, with reference to Algerian microbiological standards for livestock feeds. The reduction rates of germs in the 2<sup>nd</sup> series compared to the 1<sup>st</sup> series were as follows: total aerobic mesophilic flora (86.45%), total coliforms (96.43%), faecal coliforms (70.41). %, *Clostridium perfringens* (88.4%), yeast and mold (87.75%). The total absence of *Staphylococcus aureus* and *Salmonella spp.* was registered.

**Keywords:** pH reduction; HCl 1N treatment; feeds microbiological quality; agro-industrial by-products.

## INTRODUCTION

Animal production depends mainly on forage availability and forage quality. Forage resources in Algeria consist mainly of cereal stubble, pasture fallow vegetation, steppe rangelands, forests, maquis and some cultivated fodder (Kali et al., 2011). Cultivated forage contributes little to the feeding of herbivores (6.1% of useful agricultural area) compared to spontaneous forage plants (Bencherchali and Houmani, 2010). This would be due to several factors. On the one hand, recent years have seen climatic changes in North Africa and the Greater Maghreb. Also, three quarters of Algeria surface are subject to climatic influences: hyper-arid, arid and semi-arid, characterized by a marked summer drought (Nouaceur et al., 2013). On the other hand, the application of the agricultural policy of development in the steppe regions has caused very significant losses in terms of space and pastoral production (Nedjraoui, 2004).

Therefore, Yousfi et al. (2017) report that the forage deficit in the steppe area of Algeria (19 governorates) is around 40% on average over the period 2000-2014. This forage deficit has a negative impact on animal productivity and resulted in a massive reliance on importations of animal products such as dairy and meat products (Adem and Ferrah, 2002). According to Algerian Ministry of Agriculture and Rural Development (MARD, 2014), imports exceeded 770,000 tons of barley and 13,000 tons of oats for more than 2 million USD. Among the solutions adopted by Algerian authorities to correct

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this deficit, it is the import and the valorization of by-products (Madani et al., 2004). However, the good use of by-products in animal feed requires the control of their conservation, the knowledge of their composition, their nutritional value and the means likely to improve them (Magnier, 1991).

In Algeria, millions of tons of agro-industrial by-products are discarded, including rumen content (RC) in slaughterhouses. According to Beranger and Robelin (1978), in cattle, RCs are estimated at about 25 kg per animal slaughtered with (13.4±0.3 kg) of dry matter (DM). Every year, nearly 9 million sheep and 330,000 head of cattle are slaughtered in Algeria (Kardjadj and Luka, 2016). Faced with these numbers of slaughtered animals and a simple calculation, it can be estimated that thousands of tons of RCs would be lost each year.

Indeed, the RC is widely available as a by-product of the slaughterhouse and mainly considered as a waste creating environmental pollution (Abouheif et al., 1999; Cherdthong and Wanapat, 2013). With proper treatment and use, RCs could be a valuable source of nutrients, when included in diets for various livestock categories (Abouheif et al., 1999). The recovery of these wastes is justified for two reasons : firstly to unclog the storage areas, limit the increasing sources of pollution, and protect the population bordering public landfills against the mistrust of this waste type (Labioui et al., 2007; Chennaoui et al., 2012) ; and secondly, to recover a rich source of organic matter, which is the RC (with more than 39.86 % of DM) and other elements that may be of real interest in the feeding of livestock or in agriculture (Petersen et al., 2003; Chennaoui et al., 2012). Also, the recovery of slaughterhouse waste can help us obtain high quality fertilizer products (Chennaoui et al., 2012). However, this process must meet waste management regulations (Raabe et al., 2001).

Ruminants, through the action of their microbiota, can utilize lignocellulose that is the most abundant carbon polymer on the planet, with the rumen having a central role in releasing this vast energy store. The rumen ultimately uses lignocellulose to make products as milk and meat that are then available to humans to consume as a nutrient dense food source (Matthews et al., 2019). However, contamination of the RC during or after slaughter, by more or less pathogenic germs, always remains probable, which would require a reduction of this flora using physical or chemical means, before incorporating the RC in animal feeding.

Several previous studies reported the feeding of animals with RC. In Thailand, Seankamsorn and Cherdthong (2019) suggested that the use of RC in pellet form could be an alternative strategic supplement for Thai-native, Wagyu-crossbred cattle to enhance nitrogen balances and microorganisms. According to Mwesigwa et al. (2020), the use of RC in animal feeding is still low and limited to pigs and layers in Uganda. In Ethiopia, Gebrehawariat et al. (2016) showed that RC is a potential feedstuff for layers; it is economical and simple to practice and it is a promising feedstuff particularly during the periods of scarcity and high cost of conventional feeds.

In some contexts, the application of traditional centralized waste management solutions results in high collection and processing costs. These costs are recorded at the level of equipment and huge means of transport (Labioui and Cherkaoui, 2009). In order to minimize these costs and in the context of the valorization of slaughterhouse by-products, our study proposes the attenuation of the bacterial flora of the RC by HCl 1N treatment with a view to its valorization in animal feeding.

The microbiological quality of the RCs obtained from ruminants' slaughterhouses located in the governorates of Biskra and M'Sila was determined with the aim of incorporating them as a supplement in the feed of farm animals (ruminants, poultry, rabbits, etc.) after proposing a method to reduce their microbial load with a view to their conservation, especially since RCs are considered to be a favorable environment for microbial development (Labioui et al., 2007).

## MATERIALS AND METHODS

### Approach and methodology of study

A total of 24 RCs samples were collected, three times spaced, between February and March 2015. For each ruminant species (cattle, sheep and goats), eight samples were taken (Table 1). For each animal, two samples were collected; one of which is considered as a control (not treated with HCl 1N), while the other is treated with hydrochloric acid (HCl) 1N. The volume of the HCl 1N solution used is on average 0.8 mL/10 g of food.

**Table 1.** Summary of sampling scheme and microbiological analyzes carried out

Animal species	Number per animal species	Number of samples per animal species	Searched flora
Cattle	4	8	Total mesophilic aerobic flora (FMAT) Total and fecal coliforms
Sheep	4	8	<i>Clostridium perfringens</i> <i>Staphylococcus aureus</i>
Goats	4	8	Yeasts and molds <i>Salmonella spp.</i>

## Sampling

Random sampling of fresh RCs was done directly after slaughter and evisceration of the animals. By following the recommended aseptic rules (Konte et al., 1990), each RC sample collected was homogenized by rumen rotation movements immediately after slaughter. Then, the immediate identification of samples is carried out using an indelible marker.

The samples have been taken between 7:00 and 8:30 AM and then forwarded directly into an insulated cooler at the laboratory of microbiology and biochemistry in University of M'Sila, where they undergo microbiological treatments and tests immediately.

## Protocol and standards for microbiological analyzes

In the laboratory, the pH of RCs samples was measured by a pH meter previously calibrated on 10 g of sample (semi-liquid medium). Reading was carried out directly on the graduated scale of the apparatus. Subsequently, the dilution of the appropriate HCl to stabilize the pH to a value ( $\text{pH} \leq 2$ ) was determined, in order to attenuate the load of the possible pathogenic microorganisms and to ensure a sufficiently acidic environment and unfavorable to the microbial development. After recording the pH of each sample before and after treatment, it was found that the diluted solution of HCl 1N is the most effective in reducing the acidity to the desired value ( $\text{pH} \leq 2$ ).

The microbiological analyzes carried out (Table 1) in three dilutions in buffered peptone water were:

**Total mesophilic aerobic flora (FMAT):** whose enumeration was carried out after inoculation on ordinary nutrient agar (GNO) and incubation at 30 °C for 72 hours (AFNOR, NF 08-051, 1991).

**Total coliforms (TC) and fecal coliforms (FC):** The count is performed on VRBL agar after 48 h incubation at 37 °C for TC and 44 °C for FC (AFNOR, NF 08-060, 2009).

***Clostridium perfringens* (CP):** in sterile tubes, 1 ml of stock solutions and decimal dilutions have been introduced. These tubes were placed in a water bath for 15-20 min at 70-75 °C to destroy any vegetative forms that may be present and possibly activate the sporulated forms. Immediately at the outlet of the water bath, these tubes were cooled under tap water. Subsequently, a quantity of 18 to 20 mL of Mande Liver agar was melted and then cooled to  $45 \pm 1$  °C, it was then supplemented with 0.2 mL of iron Alum and 0.5 mL of sodium sulphite 5%; this mixture was added to each test tube. Thus, the prepared media mixed with the inoculum were gently agitated to prevent the formation of air bubbles. After solidification on bench, the tubes were incubated at 37 °C for 48 hours (AFNOR, NF 08-056, 1994).

***Staphylococcus aureus*:** isolated on Baird Parker selective medium supplemented with potassium tellurite and egg yolk, and incubated at 37 °C for 48 hours. Black colonies, shiny, curved and surrounded by an opaque zone and a clear halo were also taken into consideration (AFNOR, NF 08-057, 2004).

***Salmonella spp.*:** pre-enrichment was achieved by the addition of 25 g of the sample to 225 mL of sterile buffered peptone water in a 250 mL Erlenmeyer flask. The flask was incubated at 37 °C for 24 hours. The enrichment was carried out on 10 mL of sodium selenite broth (SFB) to which 1 mL of pre-enrichment medium was added. The tubes were incubated at 37 °C for 24 hours. The isolation was carried out on Hecktoen agar and incubated at 37 °C for 24 hours (AFNOR, NF 6579, 2002).

**Yeasts and molds:** by counting colonies grown after surface seeding and incubation at 25 °C for 2 to 5 days, on OGA medium (AFNOR, NF 08-059, 2002).

## Results interpretation

The interpretation was based on a classification into two categories: satisfactory and unsatisfactory, from a microbiological point of view and depending on a limit value " $m = M$ ". In some cases, the presence of a single organism, such as *Salmonella spp.*, is unacceptable (Di Bartolomeo, 2011). Colony count and results expression were performed according to the standard (AFNOR, NF, XP V08-102, 1998).

## Statistical analyzes

Statistical analyzes were performed using the IBM SPSS Statistics software version 21 (2011). The mean, standard deviation (SD), minimum and maximum were first calculated. Then a non-parametric test (Kruskal-Wallis test) ( $P < 0.05$ ) was performed for comparison between animal species before treatment, and a Wilcoxon test ( $P < 0.05$ ) for the comparison of microbiological quality before and after treatment. The criteria for the microbiological assessment of the results obtained come from the Algerian standards defined by the circular of March 30, 1990 (MARD, 1990) relating to the control of animal feeds.

## RESULTS AND DISCUSSIONS

### pH of RCs samples

RC samples have slightly acidic to neutral values, in the interval (5.96-7.05). The average pH value of RCs samples

is (6.61±0.57). It is comparable to that reported by Labioui et al. (2007) (6.72±0.23) for ruminants' RCs obtained from slaughterhouses in Morocco. The development of rumen microorganisms is directly dependent on the physicochemical conditions of the environment. The rumen pH value is slightly acidic, generally between (6.0 and 7.0) (Kinet, 2011). The average pH values obtained during this present study prove that rumen microorganisms before slaughter grow and ferment properly (microbial ecosystem in equilibrium), and this allows us to deduce that there is a good degradation of ingested food and therefore a good synthesis of microbial proteins and vitamins.

After slaughter and evisceration of the digestive tract, the internal balance of the rumen is interrupted and the medium becomes aerobic by promoting the multiplication of pathogenic germs, which is affirmed by Cuq (2007), reporting that the majority of pathogen microorganisms are neutrophils. Therefore, to limit their development, the chemical treatment via HCl 1N of the RCs was carried out to decrease the pH value to ( $\leq 2$ ). A similar study done by Labioui and Cherkaoui (2009), affirms that the acidification of the medium (RC) by bacteriocins produced by lactic acid bacteria, allows the destruction of pathogenic germs (FC, *Salmonella* spp. and *Clostridium* spp.) during the first week of fermentation.

### Microbiological quality of RCs before treatment with HCl 1N

Table 2 and Figure 1 summarize the microbiological quality of 12 RCs samples not treated with HCl 1N.

**Table 2.** Statistical parameters of microbial flora detected in RCs samples not treated with HCl 1N (CFU/g)

Microbial flora	Min	Mean	Max	SD
FMAT (x 10 <sup>3</sup> )	3, 65	8,86	37	9
TC (x 10 <sup>3</sup> )	1, 94	3, 93	5,5	1,1
FC (x 10 <sup>3</sup> )	0,19	2,67	4,4	1,27
<i>Clostridium perfringens</i> (x 10 <sup>2</sup> )	0,9	7,52	22,5	7,27
<i>Staphylococcus aureus</i>	0	0	0	0
Yeasts and molds (x 10 <sup>3</sup> )	0,2	0,98	2,1	0,53
<i>Salmonella</i> spp.	0	0	0	0

CFU: Colony Forming Unit, FMAT: Total mesophilic aerobic flora, TC: Total coliforms, FC: Faecal coliforms, Min: Minimal, Max: Maximal, SD: Standard deviation.

The RCs' level of contamination by (FMAT) was about 8.86 x 10<sup>3</sup> CFU/g, with a maximum of 37 x 10<sup>3</sup> CFU/g and a minimum of 3.65 x 10<sup>3</sup> CFU/g. Thus, our product is considered according to our two-class sampling plan: satisfactory or of good microbiological quality, that is lesser than the MARD specification for animal feeds, which is < 3 x 10<sup>6</sup> CFU/g.

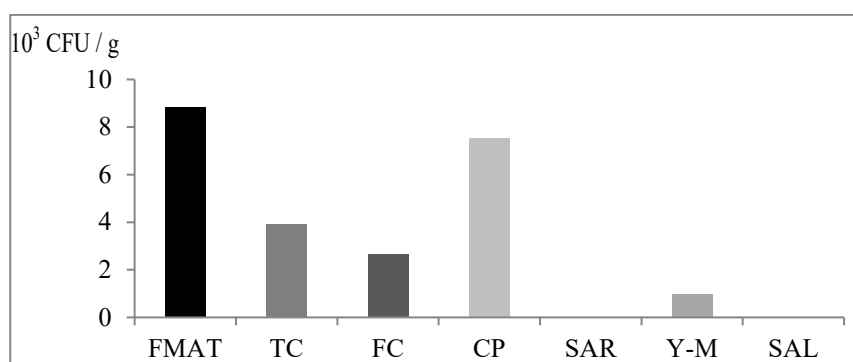
The TC ranged from 1.94 x 10<sup>3</sup> to 5.5 x 10<sup>3</sup> CFU/g with an average of 3.93 x 10<sup>3</sup> CFU/g. This value is higher than that set by MARD for animal feeds <3 x 10<sup>3</sup> CFU/g and in this case the food is considered as: unsatisfactory.

The FC were estimated at mean of 2.67 x 10<sup>3</sup> CFU/g.

*Staphylococcus aureus* and *Salmonella* spp., are absent in RCs samples.

CP was present on average by 7.52 x 10<sup>2</sup> CFU/g, and a maximum of 22.5 x 10<sup>2</sup> CFU/g. This value is higher than that fixed by the Algerian standards for animal feeds <10<sup>2</sup> CFU/g.

Yeasts and molds are estimated at an average of 0.98 x 10<sup>3</sup> CFU/g, this threshold is satisfactory by referring to Algerian standards for animal feeds (<10<sup>3</sup> CFU/g).



**FMAT:** Total mesophilic aerobic flora, **TC:** Total coliforms, **FC:** Fecal coliforms, **CP:** *Clostridium perfringens*, **SAR:** *Staphylococcus aureus*, **Y-M:** Yeasts and molds, **SAL:** *Salmonella* spp.

**Figure 1.** Means of microbial flora detected in CRs samples not treated with HCl 1N

For the microbiological quality of RCs before treatment with HCl 1N, RCs samples were considered satisfactory, because the culture results were lower than the MARD specifications for animal feeds. For comparison, our results were inferior to those found by Labioui et al. (2007) ( $15 \times 10^7$  CFU/g) and Labioui and Cherkaoui (2009) ( $15 \times 10^8$  CFU/g). The TC value is greater than that set by MARD for animal feeds ( $<3 \times 10^3$  CFU/g) and in this case the feed is considered unsatisfactory. The value obtained for *Clostridium perfringens* is higher than that set by the Algerian standards for animal feeds ( $<10^2$  CFU/g). *Clostridium perfringens* is a soil bacterium widely distributed in the environment that can contaminate forage and silage (Collins and Gracey, 1992).

The results obtained for the yeasts and molds in our present study, are satisfactory and in concordance with the Algerian standards of animal feeds ( $<10^3$  CFU/g). Comparatively, in a study conducted by Bouali (2010), *Salmonella* spp., *Clostridium* spp., yeasts and molds were totally absent in plant-based raw materials for the manufacture of livestock feed as well as finished products. In fact, the presence of molds and/or mycotoxins in animal feeds has several detrimental effects: altering the organoleptic and nutritive qualities of the fodder leading to a reduction in zootechnical performances (growth, milk yield, etc.), occurrence of various diseases (mycosis, allergies, etc.), acute or chronic intoxications related to the ingestion of mycotoxins (Boudra, 2009). In all cases, if the microorganism content (at least one microbial group) is superior to the food quality specifications, this food is considered moldy (Gafner, 2012). Thus, the RCs samples for this study, before treatment with HCl 1N were considered unacceptable.

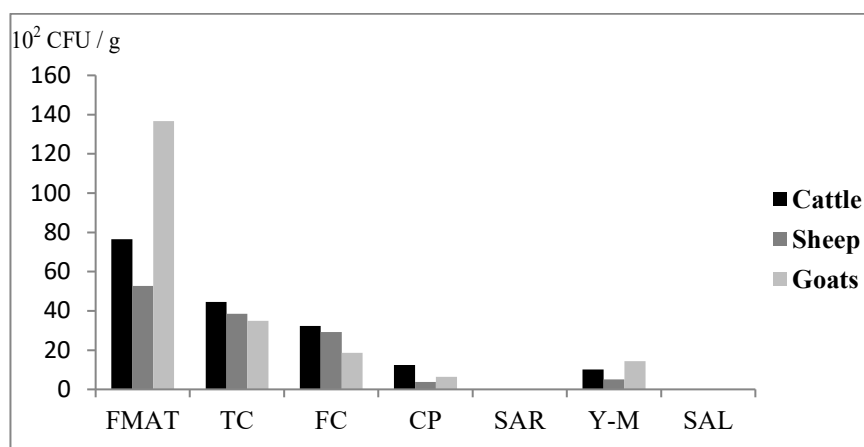
### Comparison of the microbiological quality of RCs according to animal species

It is obvious on Table 3 and Figure 2 below that there is dominance in number of FMAT, yeasts and molds in goats' RCs. The number of FMAT ( $136.62 \times 10^2$  CFU/g) recorded in goats is greater (almost double) than that of cattle's ( $76.5 \times 10^2$  CFU/g) and sheep's ( $52.75 \times 10^2$  CFU/g) RCs. The mean of yeasts and molds recorded in goats' RCs ( $14.35 \times 10^2$  CFU/g), is higher than that of cattle ( $10.22 \times 10^2$  CFU/g) and sheep ( $5.04 \times 10^2$  CFU/g). However, TC and *Clostridium perfringens* have higher mean values in cattle's RCs compared to sheep and goats.

**Table 3.** Statistical parameters and application of Kruskal-Wallis test ( $P < 0.05$ ) on microbial flora detected in RCs samples not treated with HCl 1N in different animal species (CFU/g)

Microbial flora	Cattle ( $\times 10^2$ )		Sheep ( $\times 10^2$ )		Goats ( $\times 10^2$ )		Sig.
	Mean	SD	Mean	SD	Mean	SD	
FMAT	76.5	19.63	52.75	11.84	136.62	155.73	0.365
TC	44.62	10.57	38.5	7.94	34.85	14.60	0.472
FC	32.38	8.5	29.25	8.91	18.7	17.43	0.779
<i>Clostridium perfringens</i>	12.47	8.86	3.76	5.5	6.34	5.65	0.356
<i>Staphylococcus aureus</i>	0	0	0	0	0	0	/
Yeasts and molds	10.22	1.36	5.04	3.56	14.35	5.61	0.035*
<i>Salmonella</i> spp.	0	0	0	0	0	0	/

FMAT: Total mesophilic aerobic flora, TC: Total coliforms, FC: Fecal coliforms, Sig: Statistical significance, \*Correlation is significant at  $P < 0.05$ .



**Figure 2:** Means of microbial flora detected in RCs samples not treated with HCl 1N in different animal species (CFU/g)

Kruskal Wallis test ( $P < 0.05$ ) shows that the animal species had no significant effect on the microbiological quality of RC. Consequently, RCs of the three animal species (cattle, sheep, goats) were recoverable without distinction. Nevertheless, in order to ensure better conservation, we should ensure a good drying of RCs in the open air, just after evisceration, and then treat it with HCl 1N. In fact, the very high rate of microbial mortality observed during RCs' dehydration is due, in large part, to the decrease in water activity (Cuq, 2007).

#### Microbiological quality of RCs after treatment with HCl 1N

After treatment with HCl 1N, the microbiological quality of RCs seems to be satisfactory compared to Algerian standards for livestock feeds (Table 4).

**Table 4.** Means and SD of microbial flora detected in total RCs samples after treatment with HCl 1N compared to Algerian standards for livestock feeds (CFU/g)

Microbial flora	Reference of analytical method	Specification of Algerian norm (UFC/g)	Mean and SD after treatment with HCl 1N (UFC/g)
FMAT	AN 1207	$< 3 \times 10^6$	$1.2 \times 10^3 (\pm 1.1)$
TC	AN 2812	$< 3 \times 10^3$	$0.14 \times 10^3 (\pm 0.1)$
FC	AN 2812	$< 3 \times 10^3$	$0.79 \times 10^3 (\pm 1.3)$
<i>Clostridium perfringens</i>	AN 2816	$< 10^2$	$0.87 \times 10^2 (\pm 1.38)$
<i>Staphylococcus aureus</i>	AN 2813	$< 10^5$	-
Yeasts and molds	AN 2817	$< 10^3$	$0.12 \times 10^3 (\pm 0.6)$
<i>Salmonella</i> spp.	AN 1203	Absence in 25 g	-

FMAT: Total mesophilic aerobic flora, TC: Total coliforms, FC: Fecal coliforms, SD: Standard deviation, AN: Algerian Norm

The comparison of RCs' microbial quality before and after treatment, shows that the use of the HCl 1N solution reduces the number of microorganisms to a percentage ranging from 70.41% to 96.43% (Table 5).

**Table 5.** Means of microbial flora detected in total RCs samples and application of Wilcoxon test before and after treatment with HCl 1N and microbial's decrease rates (%)

Microbial flora	Mean before treatment (CFU/g)	Mean after treatment (CFU/g)	Decrease rate (%)	Sig.
FMAT ( $\times 10^3$ )	8.86	1.22	86.23	0.002**
TC ( $\times 10^3$ )	3.93	0.14	96.43	0.002**
FC ( $\times 10^3$ )	2.67	0.79	70.41	0.003**
<i>Clostridium perfringens</i> ( $\times 10^2$ )	7.52	0.87	88.43	0.002**
<i>Staphylococcus aureus</i>	-	-	-	-
Yeasts and molds ( $\times 10^3$ )	0.98	0.12	87.75	0.002**
<i>Salmonella</i> spp. (absence in 25g)	-	-	-	-

FMAT: Total mesophilic aerobic flora, TC: Total coliforms, FC: Fecal coliforms, Sig.: Statistical significance, \*\*Correlation is significant at  $P < 0.01$ .

Wilcoxon test for two linked samples shows that there is a highly significant difference at ( $P < 0.01$ ) between RCs before treatment and after treatment with HCl 1N. This demonstrates the effectiveness of HCl 1N in attenuating the bacterial load of RC by aiming to its valorization in animal feeding.

Many studies reported the possible valorization of ruminants' RCs. According to a study conducted by Bakrie et al. (2018), the crude protein content of fermented goat's RC is much better than the rice straw, field grass, elephant grass or sugar cane top. Therefore, the fermented goat's RC, may be used as substitute for various type of forages or as an ingredient in the concentrates for various types of animals. Furthermore, the same authors reported that the cow's RC have been successfully used as feeds for cattle, goats, chickens, rabbits and fish. Also, Utomo et al. (2016) reported that the use of RC as substitute for forage (Napier grass) to ruminants decreased feed intake, increased average daily gain, and resulted in better feed conversion. However, using RC up to 67% in the diet resulted in the greater carcass percentage. Thus, authors concluded that the optimum use of RC silage is up to 67% in the diet.

Furthermore, in a study done by Makinde et al. (2017), authors concluded to the potential use of camel's RC in the diet of broilers. The result of this study showed that up to 15% use of camel's RC could be included in the diet of broilers to replace maize and groundnut cake. Beyond this level, growth parameters may be affected.

Moreover, results of an experimental study conducted by Moningkey et al. (2016) revealed that RC and sludge mixture fermented at the bacteria (*Cellulomonas* sp.) concentration of  $10^7$  CFU/g DM and 8-day incubation gave an

optimal outcome based upon its nutrient content for rabbit feed. According the same authors, the use of these wastes (RC) could also solve quality and sustainable feed availability problems and reduce environmental pollution impacts.

## CONCLUSIONS

This study has shown that the attenuation of the bacterial load of the RC via HCl 1N with a view to its valorization in animal feed, is feasible at least for the three species of domestic ruminants: cattle, sheep and goats. In addition, it appears that the lowering of the pH by a simple chemical treatment (HCl 1N) is inexpensive and easily applicable and would allow the valuation of a by-product always available at slaughterhouses, in feeding of domestic animals. Also the obvious positive economic impacts of our study are: the lowering of feeds' imports monetary charges for Algerian authorities, the coverage of summer and drought periods in animals' foods intake. In order to successfully carry out the RC valorization project in animal feed (ruminant or monogastric), it would be ideal to set up recycling units close to the slaughterhouses while taking into consideration the possibility of including RC of camelids reared in steppe and Saharan regions.

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## Conflicts of Interest

The authors declare that they do not have any conflict of interest.

## REFERENCES

1. Abouheif MA, Kraidees MS, Al-Selbood BA. The utilization of rumen content-barley meal in diets of growing lambs. *Asian-Aus J Anim Sci.* 1999; 12(8):1234-40.
2. Adem R, Ferrah A. Fodder resources in Algeria: structural deficit and regional disparities : Analysis of the forage balance for the year 2001. *Dv* 2002; 151(2):165-72.
3. AFNOR, NF, ISO 6579. Food microbiology: Horizontal method for the detection of *Salmonella* spp. 2002. Available from: <https://www.iso.org/fr/standard/29315.html>.
4. AFNOR, NF, V 08-056. Food microbiology: Enumeration of *Clostridium perfringens* by counting colonies at 45 °C. Routine method; 1994. Available from: <https://m.boutique.afnor.org/fr-fr/norme/v08056/microbiologie-alimentaire-denombrement-des-clostridium-perfringens-par-comp/fa036373/56619>.
5. AFNOR, NF, V 08-060. Food microbiology: Enumeration of fecal coliforms at 44 °C and total coliforms at 37 °C; 2009. Available from: [https://www.favv-afsca.be/laboratoires/laboratoiresagrees/\\_documents/2021-03\\_liste-meth-microbio\\_V30\\_FR.pdf](https://www.favv-afsca.be/laboratoires/laboratoiresagrees/_documents/2021-03_liste-meth-microbio_V30_FR.pdf).
6. AFNOR, NF, V 08-051. Food microbiology: Enumeration of the total aerobic mesophilic flora by counting the colonies at 30 °C; 1991. Available from: <http://www.onssa.gov.ma/images/Normes-homologues-OCTOBRE2019.pdf>.
7. AFNOR, NF, V 08-059. Food microbiology: Enumeration of yeasts and molds by counting colonies at 25 °C; 2002. Available from: <https://www.boutique.afnor.org/fr-fr/norme/nf-v08059/microbiologie-des-aliments-denombrement-des-levures-et-moisissures-par-comp/fa120539/20449>.
8. AFNOR, NF, V 08-057. Food microbiology: Enumeration of *Staphylococcus aureus* by counting colonies at 37 °C; 2004. Available from: <https://www.boutique.afnor.org/fr-fr/norme/nf-v080571/microbiologie-des-aliments-methode-de-routine-pour-le-denombrement-des-stap/fa125288/22528>.
9. AFNOR, NF, XP V08-102. Food microbiology: General rules for counting colonies and for expressing results; 1998. Available from: <https://www.boutique.afnor.org/fr-fr/norme/xp-v08102/microbiologie-des-aliments-regles-generales-pour-le-comptage-des-colonies-e/fa031737/56819>.
10. Bakrie B, Sente U, Mayasari K, Syah RF. Effectiveness of accelerator and inoculum in fermentation of goat's rumen contents as animal feed ingredients. *IOP Conf Series: Earth and Environmental Sci.* 2018; 119: 012008. Doi :10.1088/1755-1315/119/1/012008.
11. Bencherchali M, Houmani M. Forage interest for ruminants of two spontaneous forage species *Bromus*

- Madretensis* L. and *Bromus Maximus* Desf. Eur J Sci Res. 2010; 43(3):307-15.
12. Beranger C, Robelin J. Estimation of the cattle's digestive contents weight from the weight of the rumen contents. *Annales de Zootechnie*. 1978; 27(4):639-45. Available from: <https://hal.archives-ouvertes.fr/hal-00887827>.
  13. Bouali W. Contribution to the implementation of a HACCP plan in an animal feed manufacturing unit. Magister dissertation. Food Microbiology Option. University of Oran Es-Senia, Algeria, 2010; 172 p. <https://theses.univ-oran1.dz/document/TH3067.pdf>.
  14. Boudra H. Mycotoxins in fodder: a factor insidiously limiting the quality of fodder and the performance of ruminants. *Fourrages*. 2009; 199:265-80. Available from: [hal-02655830](https://hal.inrae.fr/hal-02655830) <https://hal.inrae.fr/hal-02655830>.
  15. Chennaoui M, Farid Y, Hamdani A, Mountadar M, Assobhei O. Biotransformation of slaughterhouse waste for its valorization in animal feeding. *Déchets sciences & techniques - Revue francophone d'écologie industrielle*. 2012; 61. Doi: 10.4267/dechets-sciences-techniques.2594.
  16. Cherdthong A, Wanapat M. Manipulation of *in vitro* ruminal fermentation and digestibility by dried rumen digesta. *Livest Sci*. 2013; 153:94-100. Doi: 10.1016/j.livsci.2013.02.008.
  17. Collins DS, Gracey JF. Food poisoning and meat microbiology. In *meat hygiene*, 9<sup>th</sup> edition. London: Baillière Tindall; 1992; p. 222-50.
  18. Cuq J-L. Food microbiology: The relationship between microorganisms/food/consumers. Department of Food Industry Sciences and Technologies 4th year. University of Montpellier II. Sciences et Techniques du Langue doc; 2007; p. 8-15.
  19. Di Bartolomeo M. Microbiological criteria for foodstuffs. Guidelines for interpretation. Luxembourg; 2011: 49 p. Available from: [https://qualitis.lu/wp-content/uploads/2013/07/recueil\\_criteres\\_microbiologiques.pdf](https://qualitis.lu/wp-content/uploads/2013/07/recueil_criteres_microbiologiques.pdf).
  20. Gafner J-L. The microbiological quality of animal feed. *Recherche Agronomique Suisse*. 2012; 3(5):252-57. Available from: <https://www.agrarforschungschweiz.ch/fr/2012/05/la-qualite-microbiologique-des-aliments-pour-animaux/>.
  21. Gebrehawariat E, Animut G, Urge M, Mekasha Y. Sun-dried bovine rumen content (SDRC) as an ingredient of a ration for White Leghorn Layers. *East African Journal of Sciences*. 2016; 10(1):29-40.
  22. IBM. SPSS Statistics 20 Central System User's Guide. © Copyright IBM Corporation 1989; 2011. Available from: <http://www.ibm.com/spss>.
  23. Kali S, Benidir M, Ait Kaci K, Belkheir B, Benyoucef MT. Situation of the milk sector in Algeria: An analytical approach from upstream to downstream. *LRRD*. 2011; 23(8). Available from: <https://www.lrrd.org/lrrd23/8/Kali2308cit.htm>.
  24. Kardjadj M, Luka PD. Current situation of milk and red meat industry in Algeria. *J Nutr Food Sci*. 2016; 6:516. Doi: 10.4172/2155-9600.1000516.
  25. Kinet R. Production of an anaerobic cellulolytic starter for its valorization in animal feed. Thesis of Master bio-engineer in chemistry and bio-industries. University of Liège. 2011; 108 p. Available from: [https://orbi.uliege.be/bitstream/2268/175939/1/kinet\\_ulggxabt\\_memoire-production%20d'un%20starter%20cellulolytique%20ana%C3%A9robique.pdf](https://orbi.uliege.be/bitstream/2268/175939/1/kinet_ulggxabt_memoire-production%20d'un%20starter%20cellulolytique%20ana%C3%A9robique.pdf).
  26. Konte M, Vassiliades G, Leforban Y. Biological samples for laboratory analysis. ISSN OSSO-8798.1990; 4(4). Available from: <http://hdl.handle.net/10625/15614>.
  27. Labioui H, Elmoualdi L, Benabbou Y, Ouhssine M, Elyachoui M. Treatment and recovery of waste from slaughterhouses in Morocco. *Agro solutions*. 2007; 18(1):35-40. Available from: <https://numerique.banq.qc.ca/patrimoine/details/52327/59942?docref=GfUc7I1HvLILYhsX4jeM5w>.
  28. Labioui H, Cherkaoui J. Application tests of slaughterhouse waste treated for agricultural purposes: case of wheat cultivation. *Les technologies de laboratoire*, 2009; 17. Available from: <https://revues.imist.ma/index.php/technolab/article/view/372>.
  29. Madani T, Muffok C, Frioui M. Effect of concentrate level in the ration on milk production in semi-arid Algeria. *Renc Rech Rum*, 2004 ;11. Available from: <http://www.journees3r.fr/>.
  30. Magnier L. Use of vine by-products in animal feed. E.N.S.S.A.A.-INRA. DIJON, France. *Options Méditerranéennes - Série Séminaires*. 1991; 16:89-99. Available from: <https://docplayer.fr/44964758-Utilisation-des-sous-produits-de-la-vigne-dans-l-alimentation-animale.html>.
  31. Makinde OJ, Abdullahi AM, Mohammed G. Evaluation of camel rumen content as a feed for broiler chickens. *Trakia J Sci*. 2017; 15(2):128-134. Doi:10.15547/tjs.2017.02.005.
  32. MARD. Agricultural statistics, areas and productions. Ministry of Agriculture and Rural Development Series B. Algeria; 2014. Available from: <http://madrp.gov.dz/agriculture/statistiques-agricoles/>.
  33. MARD. Circular; Control of poultry feed. Ministry of Agriculture and Rural Development. Algeria. 1990; 34 p. Available from: <https://www.ecolex.org/fr/details/legislation/circulaire-relative-au-contrôle-des-aliments-des-volailles-lex-faoc086298/>.
  34. Matthews C, Crispie F, Lewis E, Reid M, O'Toole PW, Cotter PD. The rumen microbiome: a crucial consideration when optimising milk and meat production and nitrogen utilisation efficiency. *Gut Microbes*. 2019; 10(2):115-132. Doi:10.1080/19490976.2018.1505176.
  35. Moningkey S, Junus M, Sjoefjan O, Widodo E. Nutritive value evaluation on rumen content and sludge fermented with *Cellulomonas sp.* as rabbit feed. *Int J Chem Tech Res*. 2016; 9(4):650-56. Available from: [https://sphinxsai.com/2016/ch\\_vol9\\_no4/3/\(650-656\)V9N4CT.pdf](https://sphinxsai.com/2016/ch_vol9_no4/3/(650-656)V9N4CT.pdf).



36. Mwesigwa R, Karubiu PM, King'ori AM, Onjoro PA. Extent of rumen contents use in livestock diets among farmers in Uganda. *Afric J Agri Res.* 2020; 15(2):248-55. Doi: 10.5897/AJAR2019.14652.
37. Nedjraoui D. Evaluation of pastoral resources in Algerian steppe regions and definition of degradation indicators. *IAMZ-CIHEAM Cahiers Options Méditerranéennes.* 2004; 62:239-243. Available from: <http://om.ciheam.org/om/pdf/c62/04600165.pdf>.  
Nouaceur Z, Laignel B, Turki I. Climate change in the Maghreb: towards wetter and warmer conditions on the Algerian coastline?", *Physio-Geo [Online].* 2013; 7. Published online October 25, 2013, Accessed October 25, 2013. Doi: 10.4000/physioge.3671. Available from: <http://physio-geo.revues.org/3671>.
38. Petersen SO, Henriksen K, Mortensen GK, Krogh PH, Brandt KK, Sørensen J et al. Recycling of sewage sludge and household compost to arable land: fate and effects of organic contaminants, and impact on soil fertility. *Soil Till Rers.* 2003; 72:139-52.
39. Raabe RD. The rapid composting method. University of California, US, Cooperative Extension Division of Agriculture and Natural Resources. 2001; 13-15.
40. Seankamsorn A, Cherdthong A. Dried rumen digesta pellet can enhance nitrogen utilization in Thai native, Wagyu-crossbred cattle fed rice straw based diets. *Animals.* 2020; 10(1):56. Doi: 10.3390/ani10010056.
41. Utomo R, Yusiati LM, Noviandi CT, Aryogi A, Isnandar I. Rumen contents from slaughterhouse as alternative feed for replacing forage in ruminant diets. The 17<sup>th</sup> Asian-Australasian Association of Animal Production Societies Animal Science Congress. Fukuoka, Japan. 2016; pp. 715-717. Available from: <https://repository.ugm.ac.id/id/eprint/273176>.
42. Yousfi M, Chachoua I, Bougouffa N. Contribution of rangelands to the feeding of small ruminants in the steppe and in the wilaya of Djelfa, Algeria. *LRRD.* 2017; 29(2). Available from: <http://www.lrrd.org/lrrd29/2/moha29039.html>.