Laboratory-scale ensiling of Golden Apple Snails (GAS) *(Pomacea spp)*

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Abstract

A laboratory-scale study on ensiling Golden Apple Snails (GAS) *(Pomacea spp)* was conducted in order to compare different additive to snail ratios for the preservation of GAS for several months for later use as a protein feed for fattening pigs. Mature snails were purchased, the shells and covers removed and then the flesh washed with clean water and drained before being chopped into small pieces 0.5–1 cm in size. The prepared GAS was then mixed with the additive mixture of molasses and rice bran (1:9, fresh basis) in three different proportions of additive to fresh snails: 1:1, 1:2 and 1:3 on a fresh basis, and designated MRS1, MRS2 and MRS3, respectively. Each mixture had a total weight of 6 kg after mixing, and was then divided into 6 equal parts (each of 1 kg) per treatment that were placed in plastic bags that were then sealed to avoid air contamination. Each bag was put into a 3 L plastic container, and each container included a replicate of each treatment, according to a Completely Randomized Design. Samples of GAS silage were analyzed at 0, 7, 14, 21, 28, 56, 84 and 168 days. Initially the MRS1 silage had a brown color that was somewhat darker for MRS2 and MRS3. After 7 days the color for all treatments changed to yellow-brown and each had a good smell. The color did not change further, but treatments MRS2 and MRS3 had a dark surface. Dry matter (DM) and organic matter (OM) contents decreased with ensiling time in all treatments ($P<0.05$). Crude protein (CP) remained constant in MRS1, except for a higher value at 21 days, and increased with ensiling time in MRS2 and MRS3 ($P<0.05$). The concentration of ammonia-N increased with time of ensiling on all treatments, and the highest value of 1.62 % of DM was for the MRS3 silage at 168 days. pH values fell to below 5.0 in the first 7 days of ensiling ($P<0.05$) on all treatments and then remained constant, except for treatment MRS3, in which pH increased to 5.34 at 14 days ($P<0.05$) and then remained constant up to 168 days. DM and CP contents were different among treatments at all sampling times during ensiling, due to the different ratios of FGAS to the additive mixture. pH values were not different among treatments at 0 and 7 days, but thereafter were higher in MRS3 ($P<0.05$) than in the other two silages. It was concluded that Golden Apple Snail can be successfully preserved for at least 24 weeks by ensiling with 1 kg of an additive mixture of molasses and rice bran (1:9) to 1 or 2 kg of FGAS (wet weight)

Keywords: Ensilage, additive mixture (molasses and rice bran), fresh Golden Apple Snail (FGAS), nutritive value, pH.

1. Introduction

At present, the Golden Apple Snail (GAS) *(Pomacea spp)* is considered a major pest in the rice ecosystems of Laos, especially in lowland rice fields (Douangbupha *et al*, 1998). However the GAS flesh has a high protein content (Paper I), which means it can be useful as a locally available feed resource for monogastric livestock. However, the snails are only abundant in the rainy season in Laos, and so it is important to develop ways of preserving them for use throughout the year.

Silage making is a method of preservation of high moisture materials by a controlled fermentation (McDonald *et al.,* 2002) and it has been shown that fish and their by-products can be preserved for several months, with simple processing equipment and low capital investment (Rustad, 2001) and then later used as feed for livestock (Cameron, 1962; Zaitsev *et al*., 1969; Smith, 1977). Lien *et al.* (1994) stated that ensiling can render some previously unpalatable products useful to livestock by changing the chemical nature of the feed. Fish silage has also been shown to be suitable for monogastric animals such as pigs (Perez, 1995). The main function of a silage additive is to increase the nutritional value or improve the fermentation (Ohio State University Extension, 2001). The silage additive is important for supporting microbial growth during the fermentation period. Molasses is a good, cheap additive with a high water- soluble carbohydrate content of about 700 g/kg dry matter (DM) (MacDonald *et al.*, 2002). An additive mixture of rice bran and sugar palm syrup has been shown to be suitable for ensiling small fresh water fish in Cambodia, with the nutritive value of the fish protein being retained (Phiny *et al.,* 2001). A mixture of additives can give a higher nutritive value of silage products than using a single additive. Protein-rich feeds with low energy content, such as fish waste and also FGAS should not be ensiled alone, and can be successfully ensiled when mixed with one or several energy-rich products such as rice bran and molasses (Chedly *et al.*, 1998). Molasses is available at low cost in around Vientiane City and in some districts in several provinces, where sugar factories are located.

This research focuses on the effect on silage characteristics and quality of different ratios of GAS to an additive mixture of rice bran and molasses. The aim was to evaluate the effects of ensiling GAS for several months and in a subsequent experiment to evaluate it as a protein feed for fattening pigs in the off-season.

2. Materials and methods

2.1. Location

The experiment was conducted in the laboratory of the Faculty of Agriculture of the National University of Laos (NUOL), Vientiane, from March to September 2004.

2.2 Golden Apple Snails and preparation of additive mixture

The mature snails, with a diameter of 4-8 cm, were purchased each week from local markets or collected from rice fields or ponds of farmers nearby the Faculty of Agriculture. The shells and cover of the snails were removed and then the flesh washed with clean water and drained. The flesh was then chopped into small pieces of 0.5–1.0 cm in size. The molasses and rice bran were purchased from a sugar factory and a local market, respectively, then both were weighed and mixed in the proportion of 10% of molasses to 90% of rice bran, on a fresh basis.

2.3. Mixing of additive (molasses and rice bran) and GAS.

The prepared GAS was mixed with the additive mixture of molasses and rice bran (1:9, fresh basis) in three different proportions of additive to fresh snails: 1:1, 1:2 and 1:3 on a fresh basis, and designated MRS1, MRS2 and MRS3, respectively. Each mixture had a total weight of 6 kg after mixing, and was then divided into 6 equal parts (each of 1 kg) per treatment and placed in plastic bags that were then sealed to avoid air contamination. Each bag was put into a 3 L plastic container to prevent external mechanical damage and each container included a replicate of each treatment, according to a Completely Randomized Design. All containers were kept at room temperature (mean 25 ºC).

2.4. Measurements

Each container included the three different proportions of the additive to GAS (MRS1, MRS2 and MRS3), with 6 replicates per treatment. Samples were taken at 0, 7, 14, 21, 56, 84 and 168 days after ensiling for analysis of chemical composition, including dry matter (DM), crude protein (CP) and organic matter (OM) by standard methods (AOAC, 1985), and fermentation characteristics such as pH and NH_3-N in the sample was analyzed by distillation with water and MgO, collection in 0.3 % H_3BO_3 and then titration with standard 0.1 N H_2SO_4. Physical characteristics, such as smell and color were also observed and recorded.

2.5. Statistical analysis

The data were analyzed using the General Linear Models procedure of ANOVA in the MINITAB 13.31 program (2000) to determine between treatment differences.

3. Results

3.1. Chemical composition of the GAS and additives

The analyzed chemical composition of the flesh of the fresh golden apple snail (FGAS) (Table 1) shows that the DM content (181 g/kg) was much lower than the DM of the molasses (713 g/kg) and rice bran (916 g/kg). The crude protein (CP) content of FGAS (621 g/kg) was higher than in rice bran (99 g/kg).

Table 1. Analysed chemical composition of Golden Apple Snail flesh (FGAS), molasses and rice bran (g/kg DM)

	FGAS	Molasses	Rice bran
DM	181	713	916
CP	621	24	99
Ash	149	86	131

FGAS = Fresh Golden Apple Snail.

3.2. Physical characteristics

Initially the MRS1 silage had a brown color that was somewhat darker for MRS2 and MRS3. After 7 days the color for all treatments changed to a yellow-brown and each had a good smell. The color did not change further, but the surface of treatment MRS2 and MRS3 became dark. A white colored layer appeared around the opening of the plastic bags after 28 day of ensiling, and then remained to 168 days of ensiling time.

3.3. Changes in chemical composition and pH with time of ensiling

Dry matter and OM contents decreased with ensiling time in all treatments ($P<0.05$) (Table 2; Figure 1). Crude protein remained constant in MRS1, except for a higher value at 21 days, and increased with ensiling time in MRS2 and MRS3 ($P<0.05$) (Figure 2). The concentration of ammonia-N increased with time of ensiling on all treatments, and the highest value of 1.62 % of DM was for the MRS3 silage at 168 days. pH values fell in the first 7 days of ensiling ($P<0.05$) on all treatments and then remained constant, except for treatment MRS3, in which pH increased to 5.34 at 14 days (P<0.05) and then remained at this level until the end of the study (Figure 3).

Table 2. Effect of ensiling time and ratio of GAS to an additive mixture of rice bran and molasses on the chemical composition of the GAS silage (% of DM)

Parameter	Treatment *	Time of ensiling (days)							SE	P
		0	7	14	21	56	84	168		
DM	MRS1	$^{x}55.9^{a}$	$^{x}55.8^{a}$	$^{x}55.5^{a}$	$^{x}54.3^{a}$	$^{x}54.2^{a}$	$^{x}49.8^{b}$	$^{x}49.6^{b}$	0.38	0.000
	MRS2	$^{y}48.8^{a}$	$^{y}47.9^{a}$	$^{y}45.6^{a}$	$^{y}45.3^{a}$	$^{y}44.0^{b}$	$^{y}43.4^{b}$	$^{x}44.1^{b}$	0.92	0.001
	MRS3	$^{z}39.3^{a}$	$^{z}37.5^{b}$	$^{z}37.5^{b}$	$^{z}36.8^{b}$	$^{z}36.8^{b}$	$^{z}36.1^{bc}$	$^{y}35.8^{bc}$	0.31	0.000
	SE	0.93	0.59	0.37	0.39	0.43	0.45	0.81		
	P	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
OM	MRS1	$^{y}94.8^{a}$	$^{y}94.7^{a}$	$^{y}94.6^{a}$	$^{x}94.5^{a}$	$^{x}94.4^{a}$	$^{y}93.6^{b}$	$^{y}93.4^{b}$	0.12	0.000
	MRS2	$^{x}95.7^{a}$	$^{x}95.4^{a}$	$^{y}94.1^{b}$	$^{y}94.1^{b}$	$^{x}94.4^{b}$	$^{x}94.3^{b}$	$^{x}94.2^{b}$	0.13	0.000
	MRS3	$^{y}94.8^{a}$	$^{z}93.8^{b}$	$^{x}94.9^{a}$	$^{y}93.9^{b}$	$^{y}93.9^{b}$	$^{z}91.7^{c}$	$^{z}92.2^{c}$	0.13	0.000
	SE	0.08	0.11	0.13	0.08	0.090	0.17	0.19		
	P	0.000	0.000	0.005	0.001	0.006	0.000	0.000		
CP	MRS1	$^{x}19.2^{ab}$	$^{z}18.9^{b}$	$^{z}18.9^{b}$	$^{z}19.7^{a}$	$^{z}18.0^{b}$	$^{z}18.4^{b}$	$^{z}17.9^{b}$	0.39	0.025
	MRS2	$^{y}28.6^{c}$	$^{y}33.0^{b}$	$^{y}32.8^{b}$	$^{y}36.8^{ab}$	$^{y}39.8^{a}$	$^{y}39.2^{a}$	$^{y}35.4^{ab}$	0.96	0.000
	MRS3	$^{z}33.8^{c}$	$^{x}42.4^{b}$	$^{x}44.6^{b}$	$^{x}50.3^{a}$	$^{x}45.9^{b}$	$^{x}43.4^{b}$	$^{x}43.5^{b}$	0.66	0.000
	SE	0.65	0.63	0.69	0.35	1.02	0.75	0.72		
	P	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
NH_3	MRS1	$^{y}0.48^{b}$	$^{y}0.51^{b}$	$^{z}0.51^{b}$	$^{z}0.53^{b}$	$^{z}0.61^{a}$	$^{z}0.63^{a}$	$^{z}0.51^{b}$	0.014	
	MRS2	$^{x}0.66^{b}$	$^{x}0.93^{a}$	$^{y}0.81^{ab}$	$^{y}0.80^{ab}$	$^{y}0.91^{a}$	$^{y}0.95^{a}$	$^{y}0.97^{a}$	0.041	
	MRS3	$^{x}0.75^{e}$	$^{x}0.91^{d}$	$^{x}1.10^{c}$	$^{x}1.38^{b}$	$^{x}1.39^{b}$	$^{x}1.59^{a}$	$^{x}1.62^{a}$	0.030	
	SE	0.048	0.018	0.019	0.025	0.029	0.040	0.019		
	P	0.004	0.000	0.000	0.000	0.000	0.000	0.000		
pH	MRS1	$^{x}6.60^{a}$	4.55^{b}	$^{y}4.68^{b}$	$^{y}4.65^{b}$	$^{y}4.73^{b}$	$^{y}4.64^{b}$	$^{z}4.50^{b}$	0.099	0.000
	MRS2	$^{y}6.38^{a}$	4.74^{b}	$^{y}4.87^{b}$	$^{y}4.83^{b}$	$^{y}4.93^{b}$	$^{y}4.83^{b}$	$^{y}4.80^{b}$	0.057	0.000
	MRS3	$^{x}6.57^{a}$	4.56^{b}	$^{x}5.34^{c}$	$^{x}5.25^{c}$	$^{x}5.30^{c}$	$^{x}5.41^{c}$	$^{x}5.49^{c}$	0.087	0.000
	SE	0.036	0.085	0.077	0.084	0.073	0.128	0.071		
	P	0.001	0.260	0.000	0.001	0.000	0.002	0.000		

*MRS1, etc: ratio of a mixture of rice bran and molasses (90% rice bran + 10 % molasses) to FGAS, fresh weight

^{a,b,c} *within rows, values with different superscript letters are significantly different (P<0.05)*

^{x,y,z} *within columns , values with different superscript letters are significantly different (P<0.05)*

3.4. Effect of ratio of additive mixture to GAS on chemical composition and pH

Dry matter and CP contents were different (P<0.01) among treatments at all sampling times during ensiling, due to the different ratios of FGAS to the additive mixture (Table 2). NH_3-N increased at all sampling times with increases in the proportion of FGAS in the silage, and was highest in MRS3 at 168 days. pH values were not different among treatments at 7 days (P>0.05), but thereafter were higher in MRS3 than in the other two silages (P< 0.01).

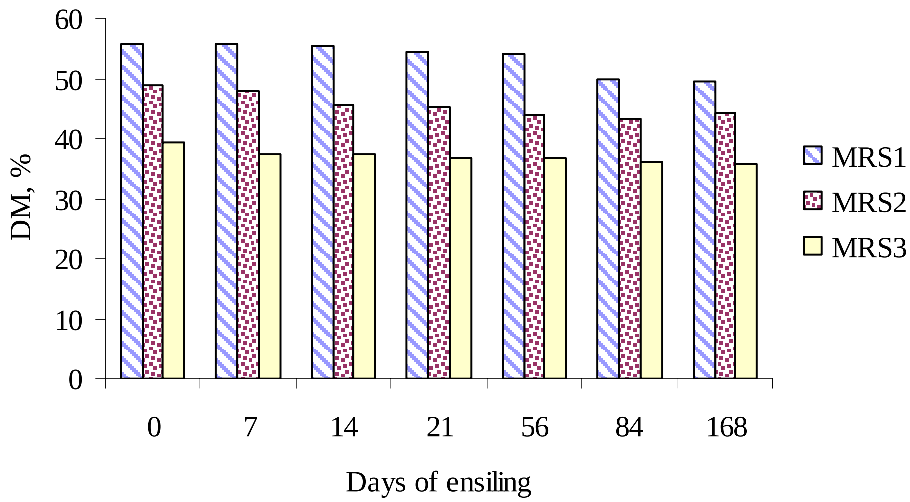

Figure 1. Change with time of dry matter content in Golden Apple Snail silage

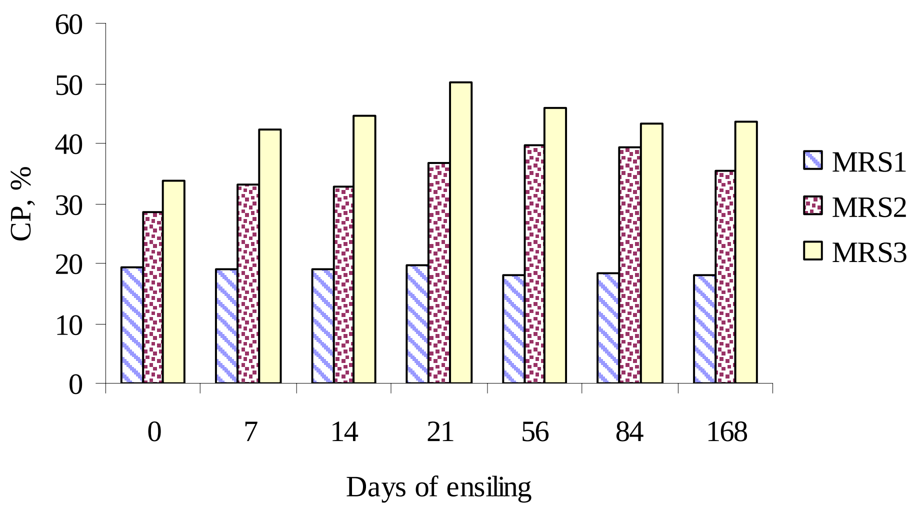

Figure 2. Change with time of crude protein content in Golden Apple Snail silage

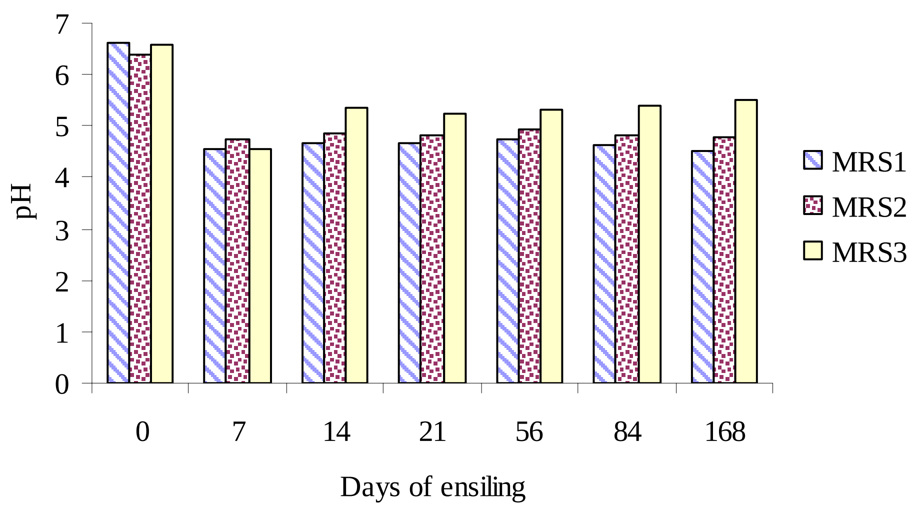

Figure 3. Change with time of pH content in Golden Apple Snail silage

4. Discussion

Golden Apple Snail flesh (FGAS) had a high content of CP (621 g/kg), which is comparable to good quality fish meal, although the DM content was low (181 g/kg), which can make ensiling more difficult unless an additive with high DM content is used (McDonald *et al.,* 2002). The rice bran and molasses contained 916 and 713 g/kg of DM, respectively, and also would have been rich in water-soluble carbohydrates (McDonald *et al.*, 2002), making the mixture an ideal additive for high-protein materials such as GAS or fish by-products. The results from the present study show that the best ratio of the additive mixture to FGAS was 1:2 (MRS2). The ensiled product had an attractive smell and also a better nutrients content and physical properties than MRS1 and MRS3. The MRS1 had a low CP content and gave off a strong smell of alcohol, from the fermentation of the starch and sugar from rice bran and molasses, respectively, while the MRS3 gave off a strong smell of ammonia after 28 days of ensiling due to the excessively high ratio of FGAS to additive (3:1), which ultimately gave a higher pH and an unpalatable silage. The DM and CP contents were different among treatments due to the different ratios of additive to snails, and the lower CP content of the MRS1 silage would mean that higher levels of inclusion in pig diets would be needed to meet CP requirements, compared to MRS2 and MRS3.

DM content decreased with ensiling time because of the conversion of starch in the rice bran and sugars in the molasses to volatile fatty acids, particularly lactic acid, which also resulted in the lower pH (Chedly and Lee, 1998). pH decreased rapidly in all treatments after only 7 days of ensiling and remained low until 168 days for MRS1 and MRS2, which had pH values of less than 5.0. However the pH of the MRS3 silage, after rapidly decreasing at 7 days, increased to over 5.3 from 14 days onwards, due to the lack of soluble carbohydrates (Carbutt, 1997). Clostridia are able to grow at these relatively high pH levels, and ferment lactic acid to butyric acid, while proteolytic Clostridia break down amino acids to amines and ammonia, which explains the increased NH_3-N and the smell of ammonia, particularly in the MRS3 treatment (McDonald *et al.,* 2002). Similar results were reported by Ngoan *et al.* (2000), who found that ratios of high-protein shrimp by-product to molasses of more than 4:1 resulted in pH levels of more than 7.0, and consequent rapid deterioration of the silage. Large amounts of rice bran that contain starch, and of molasses which is rich in sugar content (McDonald *et al.,* 1995), would support the activities of *Saccharomyces* yeasts, *Streptococcus lactis and Poteus Bacteria,* that in anaerobic conditions give alcohols, lactic acid and amines and ammonia, respectively (Potter, 1978). However, the low proportion of FGAS in MRS1 would mean a lower growth of microbial protein than in other two ratios of FGAS to additive mixture (MRS2 and MRS3). The high starch content in the rice bran and high sugar content in the molasses were fermented by *Saccharomyces* yeasts and *Streptococcus lactis* bacteria to give alcohols and then lactic acid, leading to the lower pH values of the MRS1 and MRS2 treatments (Potter, 1978).

The appearance of a white layer around the opening of the plastic bags was probably a result of a reaction of the lactic acid in the silage with oxygen from the air during sample taking, which would have caused slightly increased pH values and allowed the growth of white-colored molds (Potter, 1978).

5. Conclusions

Golden Apple Snail flesh (FGAS) can be successfully preserved for at least 24 weeks by ensiling with an additive mixture of molasses and rice bran (1:9, fresh basis) in a ratio 1 kg additive to either 1 or 2 kg of FGAS (wet weight). The 1:2 ratio of additive to FGAS resulted in a product that would be more suitable for inclusion in pig diets.

6. Acknowledgements

We are very grateful to the Swedish International Development Cooperation Agency, Department for Research Cooperation (Sida–SAREC) through the regional MEKARN Project, for the financial support of this study.

I would also like to thank the Faculty of Agriculture, National University of Laos, for allowing and helping me to carry out this laboratory-scale experiment, and the Feed Analysis Laboratory of the Department of Livestock and Fisheries of the Ministry of Agriculture and Forestry. Thanks also to the Laboratory of the Livestock

Research Center of the National Agriculture and Forestry Institute of Laos, for analysis of the samples.

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