Evaluation of three saponification methods on two types of fat as protection against bovine ruminal degradation.

F. Proaño[1], J.R. Stuart[2], Bertha Chongo[2], L. Flores[1], Magaly Herrera[2], Yolaine Medina[2] and Lucía Sarduy[2]

[1]*Facultad de Ciencias Pecuarias. Escuela Superior Politécnica de Chimborazo. Riobamba-Ecuador*
[2]*Instituto de Ciencia Animal, Apartado Postal 24, San José de las Lajas, Mayabeque, Cuba*
Email: fredyproanioortiz@gmail.com, fredyproanio@yahoo.es

To evaluate the saponification of two fat raw matters, ovine suet and residual of African palm oil (*Elaeis guineensis*), by means of the use of sodium hydroxide, potassium and calcium, with the purpose of protecting these fats from the bovine ruminal degradation, an experiment according to a completely random design, with factorial arrangement 3 x 2 was conducted. A bromatological analysis of the obtained soaps, evaluation of consistency and profitability by means of cost-benefit indicator was carried out. The variance analysis showed significant interaction among the studied factors. The dry matter was higher in the residual of palm oil soaps treated with calcium, sodium and potassium ($P \leq 0001$). Likewise, ash was higher in these soaps ($P \leq 0001$).It was higher with the potassium hydroxide. This hydroxide allowed soaps with higher ($P < 0, 0002$) protein concentrations (with both fat sources).The higher concentration of fat was in the ovine suet soaps ($P \leq 0001$).The calcium hydroxide saponification acted in an optimum way in the consistency of residual palm oil and ovine suet soaps. The cost – benefit showed higher values (1.25) in the residual palm oil soaps protected with calcium. It is concluded that the studied saponification methods (sodium, potassium and calcium hydroxide) allowed an appropriate nutrients concentration in the obtained soaps, high contribution of fat in ovine suet, adequately hardness in calcium soaps, acceptable levels of *in vitro* solubility and high profitability for calcium soaps.

Key words: *protection, fat sources, ruminant*

The supplementation with bypass fat is an alternative to increase the energetic density in fitting ration to dairy cows (Tyagi 2010) that have an energy negative balance after parturition (Gallardo 2011), because fats are inert in the rumen and digestible in the intestinal tract.

Four types of inert fats have been informed: recovered with proteins, hardness hydrogenated fats, seeds and calcium salts of fatty acids (Cabrera and Carpio 2007).

The calcium salts of fatty acids are obtained by saponification.During this process, free fatty acids are join with calcium ions. These compounds showed high melting point and solubility in pH lower at 5.5.Therefore, they are not dissociated in the rumen and dissolved in the ruminal liquid. The abomasums show pH from 2 to 2.5 that can be dissociated by means of the release of fatty acids and calcium molecules, compounds that can be digested in the intestine (Salvador *et al.* 2009).

The saponification is a chemical reaction between a fatty acid and a base, in which it is obtained the acid salt as a main product (Mateos *et al.* 1996).The bases or alkaline can be sodium salts or potassium (Bernardini and González 2009 and Bas 2001) and of calcium (InfoCarne 2008 and Herrera and Calleja 2011).All have a caustic power (García 2002 and García and Cruz 2011) and could give similar results, as protection of fatty acids against ruminal degradation.

In a previous research study, the ovine suet and palm oil residuals were characterized, abundant elements in Ecuador. The poor use of these resources contributes to the environmental pollution. The contents, relatively high, of unsaturated fatty acids present in palm oil residuals and ovine suet, could be used in the dairy cows diet, but this required to protect these fats from ruminal degradation, so they were transform into bypass fats.

The objective of this research was to evaluate three saponification methods (sodium, potassium hydroxide and calcium) on two types of fats(ovine suet and palm oil residuals), with the purpose of protecting them to the bovine ruminal degradation.

Materials and Methods

The research was carried out in the Chemical Sciences Laboratory, belonging to Facultad de Ciencias Pecuarias de la Escuela Superior Politécnica de Chimborazo, in Ecuador. This facility is located at 2,754 m o.s.l, with annual average temperature of 13.36 ºC and annual precipitation of 490.8mm, according to reports from the Meteorological Annual (ESPOCH 2011).

It was used sodium hydroxide (NaOH), potassium (KOH) and calcium ($Ca(OH)_2$) to saponificate ovine suet and residuals from the oil industry of African palm (*Elaeis guineensis*), according to completely random design with factorial arrangement 3x2 with 3 repetitions. Data were processed by means of Infostat, (version 2012).Each sample constituted an experimental unit and each one was analyzed by duplicated. The treatments were the followings:

T1: Ovine suet saponificated with KOH	T4: Oil palm residuals saponificated with NAOH
T2: Oil palm residuals saponificated with KOH	T5: Ovine suet saponificated with $Ca(OH)_2$
T3: Ovine suet saponificated with NaOH	T6:Oil palm residuals saponificated with $Ca(OH)_2$

The soaps, once obtained, were subjected to a bromatological analysis (DM, minerals, fat, protein, fiber and NFE) according to AOAC 2005.The consistency was evaluated with a non parametric scale, from 1 to 4 points. Profitability was analyzed by means of cost benefit indicator(C/B=totals income/ totals cost).

The bromatological analysis considered the percentage content of DM, from which was calculated the content of minerals, protein, fat, fiber and NFE.

For the consistency determination, punctuation scale from 0 to 4 was applied, where:

0=soaps with phase separation (watery and saponificate)

1=doughy soaps

2= soft soaps

3=half hard soaps and

4=firms or hard soaps

Duncan (1995) test was used for mean comparison in necessary cases. The non parametric measure was carried out according Conover (1999).

Results and Discussion

The statistical analysis showed the significance of the studied factors interaction (fat x saponification methods). The bromatological contents of the different studied fats are showed in table 1.

Table 1. Bromatological content of fats protected by the effect of saponification method and fat type

Variables, %	Interaction saponification method/ fat type: K (OH) Ovine suet, %	K (OH) Oil palm residuals, %	Na (OH) Ovine suet , %	Na (OH) Oil palm residuals, %	Ca (OH) Ovine suet, %	Ca (OH) Oil palm residuals, %	P	S.E
Dry matter, %	79.38f	84.21^{c}	81.16^{e}	86.25^{b}	82.89^{d}	92.98^{a}	<0.0001	0.06
Ashes, % DB	9.92e	23.92^{a}	7.74f	17.38^{c}	12.13^{d}	17.67^{b}	<0.0001	0.04
Protein, % DB	3.68a	3.37^{b}	3.10^{c}	2.44^{d}	1.87^{e}	1.81^{e}	<0.0002	0.05
Fat, % DB	86.38b	68.36^{f}	89.13^{a}	74.91^{e}	85.98^{c}	78.67^{d}	<0.0001	0.05
Fiber, %DB	0.00	3.72b	0.00	4.72^{a}	0.00	1.20^{c}	<0.0001	0.04
FNE, %DB	0.02	0.64	0.02	0.54	0.02	0.64	0.8570	0.10

P ≤ 0.05 show significant differences DB=DryBase

The soaps made with oil palm residuals (92.28% with calcium, 86.25% with sodium and 84.21 % with potassium) showed the highest (P ≤ 0001) DM content regarding to those formulated with ovine suet (calcium 82.89, sodium 81.16 and potassium 79.38%).

The higher ash concentrations (P ≤ 0001) in dry base are showed by the soaps made with oil palm residuals (potassium 23.92%, calcium 17.67% and sodium 17.38%) compared to those made with ovine suet (calcium 12.13%, potassium 9.92% and sodium 7.74%).

The potassium hydroxide, as saponificate material, allowed obtaining soaps with high protein concentration (3.68% in ovine suet and 3.37% in oil palm residuals). It fallows the sodium hydroxide (3.10%to ovine suet and 2.44% to oil palm residuals).Soaps with low protein concentration (P<0.0002) corresponded to the calcium hydroxide (1.87% in ovine suet and 1.81% in oil palm residuals).

The ovine suet soaps showed higher fat concentration(sodium 89.13%, potassium 86.38 %and calcium 85.98%) regarding the soaps made with oil palm residuals(calcium 78.67%, sodium 74.91% and potassium 68.36%).

The ovine suet soaps did not showed fiber presence. Those of oil palm residuals protected with sodium showed the higher (P<0.0001) fiber levels (4.72%), fallow by those formulated with oil palm residuals and protected with potassium (3.72%).Those made with oil palm residuals, protected with calcium (1.2%) were in last position .

Regarding to the content of nitrogen free extract, all soaps showed similar values, did not exits statistical differences (P ≥ 0.1).

Table 2 shows the results of consistency. Calcium hydroxide acted optimally and similar in soap consistency; the sodium and potassium hydroxide allowed optimal hardness, only when they acted in the ovine suet.

As reference to the profitability possible to obtain in protected fats elaboration from oil palm residuals and ovine suet, cost- benefit indicator was used , whose value are showed in table 3.

The calcic soaps of oil palm residuals showed the highest values (1.25) in cost –benefit indicator. Fallow by the calcic soaps of ovine suet (1.17) and the sodium ones,

Table 2. Consistency of ovine suet and oil palm residuals soaps

Half consistency	Oil palm residuals, PK	Ovine suet, PK
Significance	2.67	3.67
SE ± y	0.014	0.014
Raw matter	0.14	0.14
Half consistency	Oil palm residuals, PNa	Ovine suet, PNa
Significance	3.00	4.00
SE ± y	0.014	0.014
Raw matter	0.14	0.14
Half consistency	Oil palm residuals, PCa	Ovine suet, PCa
Significance	4.00	4.00
SE ± y	0.014	0.014
EE ± y	0.14	0.14

Table 3. Cost benefit of ovine suet and oil palm residuals soaps

Concept	M.U.	Ovine suet			Oil palm residuals		
		Na	K	Ca	Na	K	Ca
Rashers of ovine suet	USD	1.24	1.24	1.24	0.0	0.0	0.0
Oil palm residuals	USD	0.0	0.0	0.0	1.06	1.06	1.06
Sodium hydroxide	USD	0.42	0.0	0.0	0.23	0.0	0.0
Potassium hydroxide	USD	0.0	1.14	0.0	0.0	0.6	0.0
Calcium hydroxide	USD	0.0	0.0	0.112	0.0	0.0	0.072
Ethanol	USD	0.5	0.5	0.0	0.3	0.3	0.0
Distilled water	USD	0.25	0.25	0.25	0.25	0.25	0.25
Sodium chloride	USD	0.16	0.16	0.0	0.12	0.12	0.0
Equipment depreciation	USD	0.29	0.29	0.29	0.29	0.29	0.29
Installations depreciation	USD	0.05	0.05	0.05	0.05	0.05	0.05
Hours per process	h	14.0	12.0	18.0	14.0	12.0	18.0
Electric power cost	Total	0.28	0.24	0.36	0.28	0.24	0.36
Labor force cost	Total	0.245	0.54	0.81	0.63	0.54	0.81
Total of production cost	USD	3.435	4.410	3.112	3.210	3.45	2.892
Obtained soap	Kg	1.0	1.0	1.0	1.0	1.0	1.0
Tax to the income	USD	0.41	0.53	0.37	0.39	0.41	0.35
Packing cost	USD	0.01	0.01	0.01	0.01	0.01	0.01
Commercialization costs	USD	0.34	0.44	0.31	0.32	0.34	0.29
Total of costs	Usd	3.79	4.86	3.43	3.54	3.81	3.19
Market selling price	USD	4.0	4.0	4.0	4.0	4.0	4.0
Cost/benefit, USD	USD	1.06	0.82	1.17	1.13	1.05	1.25

formulated with oil palm residuals (1.13).The fulfilled analysis was partial because they were not considered in this values the effects in animal production.

The rule of the Standardization National Institute of Ecuador (SIN 1981) establish that the soaps made with animal fat and oils should contain, at least, 65% of dry matter, parameter that has taken as reference to consider the success of chemical process applied to oil palm residuals and ovine suet. The higher dry matter concentration in the soaps of oil palm residuals could be due to solid residuals presence that constitute the raw matter, coming from residuals of palm fruit.

The soaps of oil palm residuals showed higher ash concentration regarding to those of ovine suet, probably due to that the raw matter of oil palm residuals contain more ash than the ovine suet. Consequently, ash content showed direct relation with the ones of raw matters. It is known that ashes are an indicator of mineral content and have structural functions (P, Ca, Mg, Fe) and electrolytic (Na, K, Ca, Mg) that acted in the osmotic pressure, equilibrium and membrane permeability and tissues. It also takes part,in catalyst functions (Na, Mn, Cu, I, Se).

Because of this, is very important that food destined to animals diet contribute important amount of minerals to the ration. Murillo *et al.* (2013) informed decrease of the interval conception parturition in supplemented cows with mineral concentrate and oil palm regarding to non supplemented cows. This shows that the energy supplement cannot act if it is with minerals contribution that, by general, is relatively low in suets. In oil palm residuals do not happen in that way, in which the ash contribution was significantly higher regarding to ovine suet.

Regarding to protein concentrations, the saponification techniques that required a thermal treatment can influence in an apparent protein denatured contained in the raw matters, what is obvious in the soaps made with calcium hydroxide. This statement coincide to that informed by Pérez (2007) and Luque (2008), whose stated that in saponification process take place a protein denature , due to temperature changes and pH variation that caused the bridges break that constitutes the tertiary protein structure, making them insoluble in water.

The higher bromatological concentration of fat in the ovine suet soaps are logics if the levels of this element presents in raw matter are considered regarding to oil palm residuals. The results of this study suggest that the bromatological characteristic of the studied soaps depends on the used raw matter, especially regarding minerals, fat and fiber.

Regarding consistency (table 2), calcium hydroxide permitted the soaps optimum hardness, indistinctly of the raw matter on which it acted, can be used in saponification process, be of oil palm residuals or ovine suet. Otherwise, the use of sodium and potasium hydroxidesis not recommended for the saponification of oil palm residues, since soft soaps are get that could have conservation problems, fundamentally in the mixture of the raw materials used in the elaboration of concentrates.

The profitability calculation, in function of cost-benefit indicator, allows affirming that the fats elaboration protected from the ruminal degradation, would be profitable if it is used the calcium hydroxide as saponificate element. The lower profitabilities were observed when using potassium hydroxide, in whose values can influence, in a decisive way, the chemical reagent cost.

It is concluded that the procedures used to saponificate the raw matter studied, were appropriate. An adequate dry matter concentration, high ash concentration (especially in oil palm residuals), important energy contribution (especially in ovine suet), appropriate hardness (especially with calcium) and high profitability (especially to calcium soaps) were observed. The results suggest that the bromatological characteristic of soaps depends on the raw matter particularities, especially regarding to minerals, fat and fiber.

It is recommended the use of calcium hydroxide as saponificated element for ovine suet and oil palm residuals, because with it was obtained the best hardness of soaps and the highest profitability levels. It should be complemented the viability study of applied procedures by means of *in situ* degradability and *in vivo* digestibility.

References

ESPOCH 2011. Anuario Meteorológico. Estación Agrometeorológica de la Facultad de Recursos Naturales. Escuela Superior Politécnica de Chimborazo. 65 pp.

AOAC 2005. Official Methods of Analysis. 18 th Ed. Ass. Off. Anal. Chem. Gaithersburg, MD. USA

Bernardini, P. 2009. El aceite vegetal. Estudio de la viscosidad y densidad de diferentes aceites para su uso como biocombustible. Available: http://upcommons.upc.edu/pfc/bitstream/2099.1/9403/6/3.4.%20El%20aceite%20vegetal.pdf. [Consulted: October 8th, 2014.

Cabrera, O. & Carpio, P. 2007. Rendimiento de vacas Holstein en lactación alimentadas con grasa sobre pasante en las dietas. Available: http://www.engormix.com. [Consulted: May 12th, 2014]

Conover, W. 1999. Practical Nonparametric Statistics. John Wiley & Sons, Inc., New York

Duncan, D. B. 1955. Multiple range and multiple F tests. H Biometrics 11:1

Gallardo, M. 2011. Los nutrientes by-pass en los sistemas lecheros pastoriles: una moda o una necesidad. Infoganadero Centroamericano. LABORATORIO PROAGRO S.A. Available: http://www.proagrolab.com.ar/ampliar-articulos?id=119. [Consulted: October 26th, 2014.

García, M. 2002. Manual de prácticas de química orgánica II. Facultad de Ciencias Químicas. ESPOCH. p. 41-44

García, G. & Cruz, L. 2011. Elaboración de cuatro tipos de jabones utilizando aceites vegetales residuales de palma africana (*Elaeis guineensis*) mediante el método de saponificación. Graduated. Thesis. Universidad Estatal de Bolívar. Facultad de Ciencias Agropecuarias. Recursos Naturales y del Ambiente. p. 67-70

González, F. & Bas, F. 2001. Las grasas protegidas como fuente energética en la alimentación de vacas lecheras. Informe Agronomía y Forestal. Universidad Católica de Chile. p. 12

Herrera, F. & Calleja, F. 2011. Caracterización de las grasas de sobrepaso por medio de cromatografía de gases. Graduated. Thesis. Universidad Veracruzana. Facultad de Ciencias Químicas. Poza Rica-Tuxpan. p. 9

INEN 1981. Normas de calidad para jabones. Instituto Ecuatoriano de Normalización. Norma 839. Quito. Ecuador

InfoCarne. 2008. Alimentos para vacas lecheras. Toda la carne en internet. Available: http://www.infocarne.com/bovino/lipidos.asp. [Consulted: November 8th, 2014]

INFOSTAT 2012. Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W. (Eds.). Universidad Nacional de Córdoba, Argentina. Available: http://www.infostat.com.ar. [Consulted:October 8th,2014]

Luque, M. 2008. Estructura y propiedades de las proteínas. Texto Básico de Química General. Primera Edición. Riobamba. Ecuador. p. 23

Mateos, G., Rebollar, P. & Medel, P. 1996. Utilización de grasas y productos lipídicos en la alimentación animal: grasas puras y mezcladas. Fundación Española para el

Desarrollo de la Nutrición Animal (FEDNA). España. p.5
Murillo, B., Vera, B. & Zambrano, F. 2013. Mezcla concentrado mineral y aceite de palma sobre parámetros reproductivos en vacas lecheras en la Parroquia Colorado del Cantón Manta. Ecuador. PhD. Thesis. Medicina Veterinaria. Escuela Superior Politécnica Agropecuaria de Manabí "Manuel Félix López". Pp. 12-45
Pérez, P. 2007. Grasas de Sobrepaso. Patented Application Number: ES2005/070190
Salvador, A., Alvarado, C., Contreras, I., Betancourt, R., Gallo, J. & Caigua, A. 2009. Efecto de la alimentación con grasa sobre pasante sobre la producción y composición de leche de cabra en condiciones tropicales. Zootecnia Tropical. 27:285
Tyagi, N. 2010. Effect of bypass fat supplementation on productive and reproductive performance in crossbred cows. Trop. Anim. Health Prod. 42: 1749

Received: November 3, 2014