

# Chemical characterization and assessment of waste products from palm (*Elaeis guineensis*) oil and sheep tallow as fat raw materials protected from ruminal degradation

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The chemical characteristics, before and after filtering, of the waste products from the African palm (*Elaeis guineensis*) oil and of the sheep tallow diluted at 50, 92 and 121° C were determined. Residual water from the filtrate and the profile of fatty acids were analyzed as possible sources of protected fats from ruminal degradation. A completely randomized design was applied with three replications for each variable. Dry matter ( $P < 0.0001$ ), fat ( $P = 0.0004$ ) and fiber ( $P = 0.0018$ ) were higher respectively in the waste products of the filtered palm oil, while the protein was 3.06 % lower ( $P = 0.0019$ ) in the filtered waste oil products than in the unfiltered oil. The descriptive statistics indicated predominance of the palmitic (43.13 %) and oleic (41.47 %) fatty acids in the waste products of the filtered palm oil. In sheep tallow, diluted at different temperatures, there were higher concentrations of myristic fatty acids ( $P < 0.0001 = (2.04 \%)$ ) and the myristoleic ( $P = 0.0006$ ) respectively at 92°C. The oleic and palmitic fatty acids showed the highest coefficients of variation. The oleic presented higher concentration ( $P = 0.0048$ ) when the extraction temperature was of 50°C (36.76 %) and the linoleic was higher ( $P = 0.0003$ ) at 120°C. It is concluded that waste products from palm oil maintain the characteristics of the palm oil and that their soaps could have a performance similar to that of commercial fats from palm oil. The characteristic reported for bovine tallow could be valid for soaps produced from sheep tallow. The utilization of waste products from palm oil and sheep tallow is recommended for producing protected fats from ruminal degradation.

Key words: *fat raw materials, chemical assessment*

Dairy exploitations under grazing conditions in Ecuador are characterized by low production levels and poor reproductive indices. During the first third of lactation there is an energetic deficit (Bargo *et al.* 2002) and poor body condition due to the mobilization of fat reserves (Caldari *et al.* 2011) especially when feeds are scarce (Zambrano 2012) and the incentives required by producers are affected by low prices, poor promotion programs, technical assistance and insufficient credit (Haro 2002).

Bauman and Griinari (2001) reported that energetic supplementation with cereal grains provoked ruminal acidosis due to changes in the acetic-propionic relationship and decrease of fiber digestibility. The supply of inert lipids at ruminal level studied by Palmquist and Jenkins (1980) propitiated a decrease in fiber digestibility, voluntary intake, milk production and protein content.

Espinoza *et al.* (2010) studied the energetic supplementation to dairy cows with the utilization of protected fats from ruminal degradation. These authors reported an increase in milk yield, growth improvements, live weight of calves, body condition and live weight of cows as well as higher pregnancy rates.

Gilmore *et al.* (2011) indicated higher conception rates, decrease in the proportion of ovulation delay, increase in estrus intensity and increase of the progesterone levels.

Fat saponification through basic salts of Na, K and Ca is one of the ways of protecting ruminal fermentation (InfoCarne 2008 and Herrera and Calleja 2011), but sufficient knowledge of the chemical characteristics of

the raw materials that are going to be used is necessary.

Waste products from palm oil contain important concentrations of fats, 9.51 % in dry basis and 79.04 % humidity. These data suggest the possibility of implementing physical actions for increasing the fat content, use these waste products for the feeding of dairy cows and also, protect these fats against ruminal fermentation. For employing sheep tallow as raw material for animal feeding, its fatty content must be quantified. Up to the present, there is no report with this type of fat.

The oil industry in Ecuador has experienced an important technological and economical growth. However, the problem of environmental pollution provoked by the palm oil has not been solved. The development of the oil and cosmetological industries has decreased the practical usefulness of the sheep tallow from the meat industry that also constitutes an important source of environmental pollution.

The objective of this investigation is to fulfill the chemical characterization of the sheep tallow and of the waste products of palm oil for determining their validity as fat raw materials that can be protected from ruminal degradation and used as energetic supplement in bovine feeding.

## Materials and Methods

The study was carried out during 90 d at the Laboratory of Chemical Sciences of the Faculty of Agricultural Sciences of the Higher Technical College of Chimborazo, with the participation of specialized private laboratories.

*Chemical characterization of palm oil waste products.* The waste products from palm oil came from the extracting plant of African palm oil "TEOBROMA", located in Santo Domingo city, Ecuador. One hundred L of waste products were taken before putting them in the oxidation pools. They were transferred to the Laboratory of Chemical Sciences of the Faculty of Agricultural Sciences of the Higher Technical College of Chimborazo (ESPOCH) and homogenized in only one container. From this, three samples (two liters each) were taken for bromatological analysis (dry matter, ash, fat, protein, fiber, NFE, % dry basis) in its primary state, according AOAC (2005).

Preliminary saponification tests with palm oil waste products demonstrated that the water present in these residues prevents fat saponification. Twenty L of palm oil waste products were submitted to 24 h filtering. From residual water three samples of 250 ml each were taken for sending them to the Laboratory of Water Analysis of the ESPOCH (APHYA-AWWA-WPCF, 1985). From the filtered material three samples were taken, of two liters each, for their bromatological analysis (dry matter, ash, fat, protein, fiber and NFE, measured in per cent in dry basis) (AOAC 2005).

Later, other three samples of the filtered palm oil waste products (two liters each) were analyzed for determining the profile of fatty acids by gas chromatography (gas chromatograph equipped with an ionization detector from the Laboratory of Equipments of the National Technical College). The procedure recommended by Rodríguez *et al.* (1998) was followed. A completely randomized design was applied with three replications for the bromatological results. For the fatty acid profile, the descriptive statistics (processed in INFOSTAT, version 2012) was applied. In the necessary cases, Duncan's (1955) test for  $P < 0.05$  was utilized.

*Chemical characterization of the sheep tallow.* From the different markets of the city of Riobamba-Ecuador, 100 kg of slices of sheep tallow were purchased and sent to the Laboratory of Chemical Sciences of the FCP.ESPOCH. From this material the muscle particles contained in the slices were removed. To facilitate its cutting into pieces, slices were frozen ( $-4^{\circ}\text{C}$ ). The cut material was mixed for its homogenization. From the total volume, the bromatological analysis of three

Cuban Journal of Agricultural Science, Volume 49, Number 1, 2015 samples (1 kg each) was made (AOAC 2005) (dry matter, ash, fat, protein, fiber and NFE, % in dry basis). On considering that heating of unsaturated FA could fix hydrogen and turn the unsaturated FA into saturated (Hernández and Sastre 1999), the cut slices were submitted to thermal treatment ( $50^{\circ}\text{C}$  for three hours,  $92^{\circ}\text{C}$  two hours and  $121^{\circ}\text{C}$  for one hour), for extracting the sheep tallow. Through gas chromatography the profile of fatty acids was analyzed of three samples (1 kg each), corresponding to each level of temperature. The procedure recommended by Rodríguez *et al.* (1998) was applied.

From the sheep tallow extracted of the slices at  $121^{\circ}\text{C}$ , the bromatological analysis was performed (dry matter, ash, fat, protein, fiber and NFE, measured in per cent in dry basis), of three samples (1 kg each). For that the criteria of AOAC (2005) were followed. A completely randomized design with three replications was applied for the bromatological analysis and the descriptive statistics of the profile of the fatty acids (INFOSTAT, version 2012). Duncan's (1955) multiple range test was applied in the necessary cases.

## Results

The bromatological analysis of the palm oil waste products, before and after filtering for 24 h, is set out in table 1.

DM content was higher ( $P < 0.0001$ ) in the filtered plant oil waste products (24.46 %) regarding the unfiltered (15.61 %) whereas as content was similar at the two times of analysis. Fat content was higher ( $P = 0.0004$ ) in the waste products after oil filtering (69.50 %) than before filtering (61.56 %).

Protein concentration was lower ( $P = 0.0019$ ) in the filtered oil waste products (12.51 %) than before filtering (15.57 %). NFE concentration was also lower ( $P = 0.0018$ ) in the filtered oil waste products (2.31 %) regarding the unfiltered (9.35 %).

Finally, fiber content was higher ( $P = 0.0018$ ) after filtering (10.66 %) than before (7.69 %). The bromatological performance of the palm oil waste products in this study can be discussed from the analysis of the residual waters from the filtering.

Results from the analysis of the filtered water are shown in table 2. The different determinations were

Table 1. Bromatological analysis of the palm oil waste residues before and after filtering

Treatment Indicators	Before filtering	After filtering	SE Sign.
DM, %	15.61 <sup>b</sup>	24.46 <sup>a</sup>	0.41 $P < 0.0001$
Ash, % DB	5.83	5.01	0.55 $P = 0.3488$
Fat, % DB	61.56 <sup>b</sup>	69.50 <sup>a</sup>	0.50 $P = 0.0004$
Protein, % DB	15.57 <sup>a</sup>	12.51 <sup>b</sup>	0.30 $P = 0.0019$
Fiber, % DB	7.69 <sup>b</sup>	10.66 <sup>a</sup>	0.28 $P = 0.0018$
NFE, % DB	9.35 <sup>a</sup>	2.31 <sup>b</sup>	0.61 $P = 0.0012$

$P \leq 0.05$  indicates significant differences DB = dry basis

compared to the legal specifications of the Ecuadorian government according to the environmental legislation currently in force.

Water pH was lower (4.77) than the levels reported by Rodríguez (2011) (6.5). This implies that the methanogenic processes will not take place in these waste products and that, alternatively, are susceptible to form sulfhydic acid. The presence of phosphates was higher (16.3 mg/L) than that allowed (TULAS 2000) and the nitrate levels lower (6.10 mg/L) than normal. Total dissolved solids were higher (15400 mg/L) than those reported by Rodríguez (2011).

In table 3 are shown the FA contained in the filtered palm oil waste products. There were no short chain fatty acids (FA) in the filtered oil waste products. The palmitic (43.13 %) and oleic (41.47 %) acids were of highest presence. The same did not occur with the lauric (0.3 %) and myristic (0.9 %) with lower presence.

The bromatological analysis of the sheep tallow,

before and after fat extraction from the slices, is shown in table 4. The analysis was realized at 121° C, since at this temperature the maximum fat extraction is attained in a short time.

In 83.13 % of the total weight of the slices there was fat of possible extraction at 121° C. Only 37.25 % could be extracted at 92° C. DM content was of 99.07 % in sheep tallow extracted at 121° C, higher ( $P < 0.0001$ ) to that found in the slices (89.15 %).

Fat concentration was higher ( $P = 0.0003$ ) in the extracted fat (96.07 %) than in the slices of sheep tallow (93.49 %). In contrast, protein concentration was lower ( $P = 0.0014$  in the extracted sheep tallow (3.01 %) regarding the slices (5.4 %). However, for NFE the concentrations were similar.

In table 5 is shown the FA profile of the sheep tallow, extracted at 92, 121 and 50° C. FA concentration (capric, lauric, pentadenoic, palmitic, palmitoleic, hexadecadienoic, margaric, margaroleic, stearic and

Table 2. Quality of the water resulting from the filtering of the palm oil waste products

Determinations	Units	**Límits	Results
pH	Und.	5-9	4.77
Conductivity	mSiems/cm		8.66
Density	g/mL		1.011
ODB	mg/L	250.0	15400.0
Phosphates	mg/L	15.0	16.3
Nitrates	mg/L	40.0	6.10

\*\*Discharge limits to the public sewer system, TULAS (2000)

Table 3. Percentage of total fatty acids in the filtered palm oil waste products

Abbreviations	Fatty acid	% FA	SD	CV
C12:0	Lauric	0.30	0.10	0.3
C14:0	Myristic	0.90	0.10	0.9
C16:0	Palmitic	43.13	0.21	43.1
C18:0	Stearic	5.00	1.00	5.0
C18:1	Oleic	41.47	0.40	41.5
C18:2	Linoleic	9.20	0.46	9.2
C18:3	Linolénic	Trace	Trace	sd

Trace = content < 100 µ/g

Table 4. Bromatological analysis of sheep tallow, before and after fat extraction

Treatments Indicators	Sheep tallow in slices	Extracted sheep tallow <sup>1</sup>	SE	Sign.
DM, %	89.15 <sup>b</sup>	99.07 <sup>a</sup>	0.05	$P < 0.0001$
Ash, % DB	0.24 <sup>a</sup>	0.05 <sup>b</sup>	0.07	$P = 0.1181$
Fat, % DB	93.49	96.07	0.15	$P = 0.0003$
Protein, % DB	5.40	3.01	0.21	$P = 0.0014$
NFE, % DB	0.870	0.872	0.06	$P = 0.9830$

<sup>1</sup>Sheep tallow extracted at 121° C  $P < 0.05$  indicate significant differences

linolenic) did not depend of the dilution temperatures of the sheep tallow. Dilution of sheep tallow at 121° C influenced on the lower concentration ( $P < 0.0001$ ) of the myristic (2.04 %) FA. At the same time it was demonstrated that at lower extraction temperature, the concentration was higher. Thus, on extracting the sheep tallow at 50° C greater concentration (3.15 %) was observed. The myristoleic FA had higher concentration ( $P = 0.0006$ ) in sheep tallow diluted at 92° C (1.06 %). The lowest concentration was at 50° C.

## Discussion

The decrease of the percentage of minerals, protein and NFE in palm oil waste products after a 24 h filtering process could possible be due to the fact that such elements were present in the water eliminated with the filtration. These waters could have a pollution potential determined by pH lower than the standards internationally allowed (OMS 2008). For each ton of virgin oil produced, it has been reported that from two

Table 5. Percentage of the total fatty acids in sheep tallow extracted at different temperatures

Treatments Indicators	92°C	121°C	50°C	SE Sign.
C10:0 capric	Trace	0.13	0.11	0.01 P=0.3153
C12:0 lauric	Trace	0.07	0.05	0.03 P=0.5579
C14:0 miristic	2.52 <sup>b</sup>	2.04 <sup>a</sup>	3.15 <sup>c</sup>	0.05 P<0.0001
C14:1 miristoleic	1.06 <sup>c</sup>	0.90 <sup>b</sup>	0.57 <sup>a</sup>	0.04 P=0.0006
C15:0 pentadedilinic	0.73 <sup>b</sup>	0.81 <sup>b</sup>	0.58 <sup>a</sup>	0.04 P=0.0148
C15:1 5-pentadecenoic	Trace	0.22	0.23	0.04 P=0.8555
C16:0 palmitic	22.32	22.25	20.23	0.88 P=0.2422
C16:1 palmitoleic	0.71	0.66	0.99	0.27 P=0.6719
C16:2 hexadecadienoic	Trace	0.37	0.33	0.02 P=0.3394
C17:0 margaric	1.77	1.75	1.43	0.20 P=0.4637
C17:1 margaroleic	0.88	0.72	0.72	0.09 P=0.3889
C18:0 estearic	34.04	33.11	32.72	1.01 P=0.6592
C18:1 oleic	33.71 <sup>a</sup>	33.10 <sup>a</sup>	36.76 <sup>b</sup>	0.51 P=0.0048
C18:2 linoleic	0.92 <sup>a</sup>	2.33 <sup>b</sup>	1.14 <sup>a</sup>	0.12 P=0.0003
C18:3 linolenic	1.33	1.54	0.99	0.21 P=0.2596

$P \leq 0.05$  indicates significant differences

The pentadedilinic FA showed similar concentration on its extraction at 92°C (0.73 %) and 121° C (0.81 %). These concentrations were lower ( $P = 0.0148$ ) when the extraction temperature was of 50° C.

The oleic FA was present in higher ( $P = 0.0048$ ) concentration when the extraction temperature was 50° C (36.76 %). However, the concentration was similar at higher extraction temperatures. Extraction at 121° C allowed higher ( $P = 0.0003$ ) concentration of the linoleic FA showing that lower temperatures could not influence on its concentration.

The capric, lauric, 5-pentadecenoic and hexadecadienoic FA were present in minimum levels in the sheep tallow extracted at 92° C. Their concentrations were increased in the sheep tallow extracted at 121° C. FA with higher concentrations were stearic, oleic and palmitic.

Capric, lauric, miristoleic, pentadedilinic, palmitic, hexadecadienoic, margaic, stearic, linoleic and linolenic FA were found in lower concentrations in sheep tallow extracted at 50° C regarding that extracted at 121°C. In contrast, miristic, 5-pentadecenoic, palmitoleic and oleic FA showed higher concentrations.

to three tons of residual waters are originated. These residual waters have high biological and chemical oxygen demand for their treatment (Estrucplan 2013). In this demand an important role could be played by the presence of nitrates that, in this investigation, were at levels of 6.1 ml/L. These, in turn, could indicate the existence of water soluble protein in the water filtered from the palm oil waste products, which could be the reason of the protein content decrease found in the bromatological analysis between the initial state and after the filtering.

The palm oil waste products were rich in unsaturated FA, a characteristic also found in other studies related to the palm oil (Vargas and Zumbado 2003). This oil is widely used for the preparation of fats protected from ruminal degradation, especially with calcium salts (InfoCarne 2008). The presence of unsaturated FA allow proposing the elaboration of soaps from palm oil waste products, since these could have a performance similar to commercial fats available at the international market.

DM concentration found in sheep tallow is consistent with other reports (Ibarra *et al.* 2008) although these referred to tallow in general and not specifically to sheep tallow. This suggests that the characteristics reported

for bovine tallow (Chacha, 2011) could be valid for sheep tallow.

The protein content of the sheep tallow is relatively high, possibly because of the presence of conjunctive tissue, extracellular liquid, polysaccharide molecules, adhered proteins, collagen fibers, elastin and glycoprotein covering reported by Brandan *et al.* (2008). This peculiarity determines that protected fats produced with sheep tallow will have a protein, as well as energetic content, anticipated by the fat contribution.

Results obtained in this study, regarding the FA concentrations in sheep tallow did not agree with other papers (Vargas and Zumbado 2003 and Pérez 2007). Possibly, differences found are maybe due to the tallow type and age of the animals at slaughter. This aspect could make us think that younger animals allow higher concentrations of lauric, palmitic and linolenic FA. It must be considered that in Camal Municipal de Riobamba animals younger than six months are not slaughtered.

Taking into account the concentration values of FA of sheep tallow extracted at 50° C (table 5) and if they are compared to those of the sheep tallow extracted at 121° C, it could be stated that the lowest concentration of the unsaturated FA results at the expense of the increase in the proportion of myristic FA (3.15 %), 5-pentadecenoic (0.23 %), palmitoleic (1 %), margoleic (0.72 %) and oleic (36.4 %). This coincides with Hernández y Sastre (1999), though an eventual extraction of sheep tallow at 50° C would be not very practical. At this thermal levels FA saturation will occur, whose fusion point is low, according to Mondragón *et al.* (2005).

It is concluded that palm oil waste products require a prior filtering process and the sheep tallow must be extracted at 121° C before its use as raw material protected from ruminal degradation.

In view that the filtered palm oil waste residues were rich in unsaturated FA, it can be assumed that these maintain the characteristics of the palm oil from which they came. Its soap could have a performance similar to that of commercial fats from the available palm oil in the market, an aspect that must be studied. The bromatological characteristics found in the sheep tallow suggest that those reported for the bovine could be valid for soap produced with sheep tallow. This is an aspect that must be investigated.

Since the filtered palm oil waste products, the same as those from sheep tallow, are raw materials fit to be turned into protected fats from ruminal degradation, its use for this purpose is recommended. In addition, since the residual water from the filtration of the palm oil waste residues would have a pollutant potential due to the presence of minerals, protein and NFE, the study of biotechnological applications for animal feeding is recommended.

## References

- AOAC 2005. Official Methods of Analysis. 18 Ed. Ass. Off. Agric. Chem. Gaithersburg. EEUU
- APHA-AWWA-WPCF.1985. Standard Methods for the examination of water and wastewater. Quality of water. Environmental protection Agency.16 th Ed. Washington, D. C. Pp. 3-1325
- Bargo, F., Muller, L.D., Delahoy, J.E. & Cassidy, T.W. 2002. Performance of high producing cows with tree different feeding systems combining pasture or total mixed rations. *J. Dairy Sci.* 85: 2959
- Bauman, D. & Griinari, J. 2001. Regulation and nutritional manipulation of milk fat: low-fat milk syndrome. *Livest. Prod. Sci.* 70:15
- Brandan, N., Llanos, C., Barrios, M. B., Escalante, A. P. & Ruíz Díaz, D. A. 2008. Proteínas Plasmáticas. Nota técnica. Facultad de Medicina. Universidad Nacional del Nordeste. Pp. 2-6
- Caldari, C., Lock, A.L., Staples, C.R. & Badinga, L. 2011. Performance, metabolic, and endocrine responses of periparturient Holstein cows fed 3 sources of fat1. *J. Dairy Sci.* 94:1500
- Duncan, D. B. 1955. Multiple range and multiple F tests. *Biometrics* 11:1. Harvey, W.R. Mixedmodelleast.
- Chacha, H. 2011. Proyecto de factibilidad para crear la Empresa Agrícola, Productora y Comercializadora de Fruta de Palma Africana en el Cantón Ventanas-Ecuador. Tesis de Grado. Escuela Superior Politécnica de Chimborazo. Pp. 19-23
- Espinoza, V. J. L., Ortega, P. R., Palacios E. A. & Guillén T. A. 2010. Efecto de la suplementación de grasas sobre características productivas, tasas de preñez y algunos metabolitos de los lípidos en vacas para carne en pastoreo. *Arch. Med. Vet.* 42:25
- Estrucplan. 2013. Impactos ambientales y actividades productivas. Aceites y grasas vegetales. Available en www.estrucplan.com.ar. [Consulted: 14/11/2014]
- Gilmore, H.S., Young, F. J., Patterson, D.C., Wylie, A.R.G., Law, R.A., Kilpatrick, D.J., Elliott, C.T. & Mayne, C.S. 2011. An evaluation of the effect of altering nutrition and nutritional strategies in early lactation on reproductive performance and estrous behavior of high-yielding Holstein-Friesian dairy cows. *J. Dairy Sci.* 94:3510
- Haro, R. 2003. Informe Sobre Recursos Zoogenéticos en Ecuador. Ministerio de Agricultura y Ganadería. Subsecretaría de Fomento Agro productivo. Quito-Ecuador, Pp. 26-77
- Hernández, M. & Sastre, A. 1999. Tratado de nutrición. Ediciones Díaz de Santos. Madrid. Pp. 500-501
- Herrera, F. & Calleja, F. 2011. Caracterización de las grasas de sobrepaso por medio de cromatografía de gases. Tesis de grado. Universidad Veracruzana. Facultad de Ciencias Químicas. Ingeniería Química. Poza Rica-Tuxpan. p. 9
- Ibarra, M., Aranda, J., Armendáriz, R., Del Bosque, A., Gutiérrez, Hernández, H., Martínez, J., Sánchez, F. & Zárate, P. 2008. Dinámica del zacate buffel establecido con diferentes densidades de siembra. Memorias XXXVI Reunión Anual de la Asociación Mexicana de Producción Animal A. C. Facultad de Agronomía. Universidad Autónoma de Nuevo León. Escobedo, N.L., México. p. 266
- InfoCarne. 2008. Alimentos para vacas lecheras. Toda la carne en internet. Available en: <http://www.infocarne.com/>

- bovino/lipidos.asp. [*Consulted*: 8/10/2014]
- INFOSTAT 2012. Di Rienzo, J.A., Casanoves, F., Balzarini, M.G., Gonzalez, L., Tablada, M., Robledo, C.W. (Eds.) Grupo InfoStat. Universidad Nacional de Córdoba, Argentina. *Available* en: <http://www.infostat.com.ar>. [*Consulted*: 8/10/2014]
- Mondragón, C., Peña, L. & Sánchez, M. 2005. Química orgánica. Editorial Santillana. Bogotá. Pp. 176-180
- OMS. 2008. Contaminación de las aguas. Degradación medioambiental. Problemas del medio ambiente. Actividades contaminantes del agua y efectos perjudiciales. *Available* en: <http://html.rincondelvago.com/contaminacion-de-las-aguas.html>. [*Consulted*: 18/10/2014]
- Palmquist, D. L. & Jenkins, T. C. 1980. Fat in lactation ration: Review. *J. Dairy Sci.* 63: 1-14.
- Perez, P. 2007. Grasas de Sobrepasso. Patente. Application Number: ES2005/ 070190
- Rodríguez, J. R., Belarbi, E.L, García, J.L. S. y López, D. A. 1998. Rapid simultaneous lipid extraction and transsterification for fatty acid analyses. *Biotech. Tech.* 12:689
- Rodríguez, P. D. 2011. Aislamiento e identificación de cepas microbianas como potenciales agentes para biorremediación de efluentes en una extractora de palma aceitera. Tesis de Grado en Ingeniería en Biotecnología Ambiental. ESPOCH. Riobamba – Ecuador. Pp. 22-76
- TULAS. 2000. Norma de calidad ambiental y de descarga de efluentes: recurso agua. República del Ecuador. Registro Oficial
- Vargas, E. & Zumbado, M. 2003. Composición de los subproductos de la industrialización de la palma africana utilizados en la alimentación animal en Costa Rica. Tesis de grado. Universidad de Costa Rica. 105 pp.
- Zambrano, G. D. 2003. Utilización de los principales subproductos agroindustriales del Trópico Húmedo Ecuatoriano en la Alimentación del ganado lechero. PhD Thesis. Universidad de Granma. Cuba. 145 pp.

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