FEED EVALUATION OF HEAT, CHEMICALLY OR BIOLOGICALLY TREATED *Jatropha curcas* MEAL AS NON TRADITIONAL FEED.

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ABSTRACT

A study was conducted to determine the effect of treating Jatropha *curcas* meal with heat (JMH), biologically with lactobacillus bacteria (JMB), or chemically with isopropanol (JMI) on its anti-nutritive compounds in order to induce *Jatropha curcas* meal in ruminants feeds to replace part of the costly imported soybean meal. *In situ* trial was also conducted to evaluate degradability of dry matter (DM), organic matter (OM) and crude protein (CP) in the rumen of two canulated male buffaloes fed rice straw and concentrate feed mixture. The experimental concentrate feed mixture (CFM), contained soybean meal to be replaced with untreated Jatropha meal (JMU) by 0%, JMU (CFM^0), 25% JMU (CFM^1), 50% JMU (CFM^2) and 75% JMU (CFM^3), or heated Jatropha meal (JMH) 25% (CFM^4), 50% JMH (CFM^5) and 75% (CFM^6) or chemical Jatropha meal (JMI) 25% (CFM^7), 50% JMB (CFM^8) and 75% (CFM^9), or biological Jatropha meal (JMB) 25% (CFM^{10}), 50% JMI (CFM^{11}) and 75% JMB (CFM^{12}) of Soybean meal. Treatment JM with bacteria increased both CP and ash content, while CF content was decreased. Meantime, treatment Jatropha meal with heat (JMH) decreased CP. Other treatments had almost similar CF content. All treatments, showed a positive effect in decreasing concentration of anti-nutritive compounds. The biological treatment with bacteria resulted in the highest decrease of anti-nutritive compounds. Meanwhile heat treatment had the least effect in decreasing anti-nutritive compounds. Rations with bacteria treated JCM had highest DM and OM degradability values, as compared with other treatments. On the other hand, rations with isopropanol treated JMI, had highest CP degradability. Effective degradability ED (%) of DM and OM were highest for ration contained bacteria treated JMB. While, no significant differences were detected among rations for EDCP.

Under the conditions of the present experiment, it could be concluded that the bacterial treated JCMB could replace up to 75% of the soybean meal in the CFM. However, including Jatropha meal (JM) in ruminant rations still needs more investigation to study its effect on animal performance and its residual effect in milk and meat.

Keywords: Jatrofa curcas meal, biological treatment, chemical treatment, heat treatment, antinutritional factors and *in situ* degradability.

INTRODUCTION

In Egypt there is a problem of shortage of protein sources used for animal feed, which is caused by the expensive imported soybean meal. Therefore, there is a need to evaluate alternative protein sources to alleviate the shortage problem. *Jatropha curcas* is a tropical plant (a shrub or small tree) which can be set up on eroded lands under harsh climatic conditions (Munch and Kiefer 1989). The seed which weighs about 0.75g contains 30-32% protein and 60-66% lipid (Liberalino *et al* ., 1988), indicating good nutritional value. The meal remaining after oil extraction contains high protein

content (approximately 45-50%) and therefore would be of interest for livestock producers as feed supplement (Ahmed and Adam., 1979) . The major problem with using Jatropha meal is the high level of antinutritional compounds like trypsin inhibitor activity, phytate, saponins and lectins in the meal. These compounds, can be mitigated by various treatments. However, this is attributed by most workers to the presence of trypsin inhibitor activity (Reddy and Pierson, 1994) and lectins (Komarova *et al.*,1995). Aderibighe *et al* ., (1997) reported that heat treatment can be used to inactivate trypsin inhibitor and to increase *in vitro* rumen protein degradability of Jatropha meal (JM). The other anti-nutritional compounds (phytate, saponins and lectins) could not be decreased using heat treatment.

Several advantages are favoring Jatropha seed to be grown in Egypt such as limited water requirements, high seed yield in new reclaimed soils and good source for oil which can used be as green fuel for diesel engine. The purpose of this study was to investigate the effect of biological treatment with bacteria, heat treatment and chemical treatment with isopropanol on degrading anti-nutritional compounds in Jatropha curcus meal (JCM) and their effect on chemical composition and degradability of different nutrients of concentrate feed mixtures with different levels of untreated and treated Jatropha curcus meal (JCM).

MATERIALS AND METHODS

The experimental work of the present study was conducted at Ismalia Experimental Unit, Animal Production Research Institute, Agricultural Research Center.

Detoxification methods
Heat treatment:
Jatropha curcas meal left after extraction of oil, was heated in boiling water for 15 min to inactivate the anti-nutritional compounds (Broderick, and Graig 1980). Treated sample was air dried at room temperature (Gorrill *et al* .,1974), then stored in plastic containers until used.

Lactic acid bacteria (LAB) treatment:
Jatropha meal was treated with *Lactobacillus acidophilus* (International, Inc.) at the rate of 1g/100kg (JM), stored in plastic containers for 21 days at room temperature, then dried to about 6% moisture and was ground to pass a 2 mm screen.

Isopropanol (70%) treatment
Jatropha meal was sprayed by aqueous solution of isopropanol at the rate of 10% (w/w) to inactivate anti-nutritional compounds, then stored in plastic containers for 21 days at room temperature. The treated JM was aerated, then ground to pass a 2 mm screen, as described by Medina and Gonzalez (1990). .

Anti-nutritional compounds analysis:
Trypsin inhibitor activity was determined essentially in untreated and treated Jatropha meal samples, according to Smith *et al* (1980). Analysis of

Lectin content was conducted by haemagglutination assay described by Gordan and Marqardt (1974). Total saponin (triepennid and steroidal) content was determined using a spectrophotometric method described by Hiai *et al.*, (1976). Phytate content was determined by a colorimetric procedure described by Vairtrash and Laptera (1988). Total phenols, tannins and condensed tannins were determined by colorimetric methods as described by Makker *et al* ., (1998 a&b).

Thirteen concentrate feed mixtures (CFM's) were formulated to be iso-nitrogenous iso-energetic, through replacing soybean meal contained in the concentrate feed mixture (CFM*), with 25, 50 or 75% of untreated Jatropha meal JMU, for $CFMU^1$, $CFMU^2$, $CFMU^3$, respectively. Mixtures of (CFM*), where soybean meal was replaced with 25, 50 or 75% of heated JCMH, for $CFMH^1$, $CFMH^2$, $CFMH^3$ mixtures, respectively, or 25, 50 or 75% of treated meal with Isopropanol JCMI, for $CFMI^1$, $CFMI^2$, $CFMI^3$ mixtures, respectively, or 25, 50 or 75% of treated meal with lacto bacillus bacteria JCMB, for $CFMB^1$, $CFMB^2$, $CFMB^3$ mixtures, respectively. Representative samples of different concentrate feed mixtures, were analyzed according to A.O.A.C, (`1999). Chemical composition of 13 (CFM's) are shown in Table (1).

Table (1): Chemical composition of experimental concentrate feed mixtures (on dry matter basis).

Experimental concentrate feed mixtures	Chemical composition (%)					
	OM	CP	CF	EE	NFE	Ash
CFM^0	91.42	14.57	6.75	3.79	65.31	8.58
$CFMU^1$	90.43	14.47	6.88	3.82	65.26	9.57
$CFMU^2$	89.84	14.54	6.92	3.81	64.57	10.16
$CFMU^3$	89.68	14.52	6.93	3.90	64.13	10.32
$CFMH^1$	90.37	14.58	6.94	3.90	64.95	9.69
$CFMH^2$	90.45	14.59	6.97	3.98	64.91	9.55
$CFMH^3$	90.38	14.52	7.03	3.97	64.86	9.62
$CFMB^1$	90.28	14.63	6.68	3.89	65.08	9.72
$CFMB^2$	90.22	14.74	6.62	3.74	65.12	9.78
$CFMB^3$	90.18	14.80	6.55	3.65	65.18	9.82
$CFMI^1$	90.17	14.53	6.74	3.86	65.04	9.83
$CFMI^2$	90.08	14.47	6.70	3.81	65.10	9.92
$CFMI^3$	90.03	14.42	6.67	3.76	65.18	9.97

*CFM Concentrate feed mixture consisted of 11% soybean meal, 40%wheat bran, 39% corn , 3% rice bran, 4% molasses, 2% limestone and 1% salt.
*$CFMU^1$: CFM with 25% of JMU *$CFMU^2$: with 50% of JMU * $CFMU^3$: with 75% of JMU
* $CFMU^1$: CFM with 25% of JMH *$CFMU^2$: with 50% of JMH * $CFMU^3$: with 75% of MH
* $CFMU^1$: CFM with 25% of JMB *$CFMU^2$: with 50% of JMB * $CFMU^3$: with 75% of MB
* $CFMU^1$: CFM with 25% of JMI *$CFMU^2$: with 50% of JMI * $CFMU^3$: with 75% of JMI

Degradability of different nutrients

Nylon bags technique was used to determine degradability of DM, OM and CP for CFM,s degradability as described by Mehrez *et al* ., (1977). Two polyester bags with poor size of 45um were used for each incubation time for each of the 13 treatments. Approximately 5g of air dried CFM"s were placed

in each bag. Two canlated male buffaloes were used to determine degradability of different concentrate feed mixtures. Animals were fed 1/3 of their requirements from rice straw and 2/3 from CFM. All bags were incubated in the rumen of each animal, then they were withdrawn after 3,6,12,24,48 and 72h , rinsed in tab water until the water became clear , then they were squeezed gently. Microorganisms, attached to the residual sample were eliminated by freezing at -20C (Kamel *et al* .,1995). Zero- time loses (a) were determined by washing 2 bags in running water for 15min . The degradability kinetics of DM, OM and CP were estimated (in each bag) by fitting the disappearance values to be equation $P= a+b \, (l-e^{c})$ as proposed by Ørskov and McDonald (1979), where P represents the disappearance after time I, least squares estimated of soluble fractions are defined as the rapidly degraded fraction (a), slowly degraded fraction (b) and the rate of degradation (c). The effective degradability (ED) for tested rations were estimated from the equation of McDonald (1981), $ED= a+bc \, / \, (c+k)$, where k is the ruminal solid out flow rate was assumed to be (0.05 /h for concentrate) under feeding conditions in this study.

Statistical analyses

Collected data were subjected to one way analysis of variance as described by Steel and Torrie (1980). Significant differences among means were carried out using LSD test according to Duncan (1955). Statistical processes were carried out using the General Linear Models adapted by SAS (2000) for PC.

RESULTS AND DISCUSSION

Chemical analysis of untreated and treated Jatropha meal .

Treating JCM with lactobacillus (Lac) resulted in a decrease in CF content by about 18.8%, meanwhile other treatments had quite similar CF content. (Table2). On the other hand, CP content was increased by about 6.8% with (Lac) treatment, while other treatments resulted in a decreased in CP content by 1.63% and 1.84% with heat and isopropanol treatments, respectively (Table 2). Ash content was increased by about 4% with biological treatment by bacteria (Lac) treatment.

Table (2): Chemical composition (%) of untreated and treated Jatropha meal (on DM basis).

	Untreated	Treated		
	JM	JMH	JMB	JMI
OM	92.76	92.87	92.48	92.58
CP	40.83	40.17	43.60	40.08
CF	10.77	11.24	8.25	10.42
EE	9.45	10.33	9.21	9.52
NFE	31.71	31.03	31.92	32.56
Ash	7.24	7.13	7.52	7.42

* JM :untreated Jatropha meal   *JMH : Treated Jatropha meal with heat
*JMB :Treated Jatropha meal with Bacteria * JMI : Treated Jatropha meal with Isopropanol

Data in Table (3), showed that all treatments had a positive effect in decreasing concentration of anti-nutritive compounds, which are considered as inhibitors and had negative effect on appetite (Ahmed and Adam, 1979 and Hajos *et al.*, 1995). Bacteria treatment with lactobacillus (LB) decreased concentration of Trypsin inhibitors and lectin by about 82% and 86.7%, respectively. Meanwhile, heat treatment decreased the concentration of trypsin inhibitor and lectin by about 75.54% and 83%, respectively. On the other hand, aqueous mixture of isopropanol was found to be an effective treatment in improving JCM as it decreased concentration of trypsin inhibitor and lectin by about 61 % and 78 % , respectively.

Table (3) : Concentration of anti-nutritional compounds of untreated and treated Jatropha meal

	Untreated	Treated		
	JM	JMH	JMB	JMI
Trypsin inhibitor mg/g	23.30	8.84	4.20	5.70
Lectin mg/ml^{-1}	55.41	12.17	7.35	9.42
Phytate g/100g	6.50	3.40	2.75	4.70
Saponnin %	4.50	3.50	2.40	3.90

These results are in agreement with White *et al.*, (1989) and Hajos *et al.*, (1995) who reported that heat treatment has a positive effect by reducing trypsin inhibitor and lectin concentration in JCM. In addition, phytic acid concentration was decreased. Meanwhile, saponins concentration of JCM was less affected by the different treatment methods. These results agree with those of Reddy and Pierson (1994), Aderibigbe *et al.*, (1997) and El-Shennawy , (2005) who reported that saponins was the lowest anti-nutritional compound affected with different treatment methods. This means that treatment with lactobacillus (LB) and isopropanol had higher effect on reducing anti-nutritional compounds as compared with heat treatment, which had lower effect. These results are in agreement with the findings of Aderibighe *et al.*, (1997) who reported that heat treatment has a limited effect on lowering levels of toxicants compared to treatment with lactobacillus bacteria which was more effective to decrease anti-nutritional compounds than the heat treatment (Vesela *et al* ., 2002). Mean time the heat treatment was the lowest to decrease Trypsin inhibitors and lectin content as compared with both biological and chemical treatment.

Degradation kinetics
Estimates of ruminal degradation constants (a,b and c) with rates of DM, OM and CP disappearance of concentrate feed mixtures (CFM's) are presented in Table (4). It illustrated that washing loss fraction (a), degradable fraction (b), rate of degradation (c) and effective degradability (ED) of DM and OM were less (P<0.05) for untreated (JCMU) with 50% & 75% of JMU) levels as compared with the control mixture (CFM).

Table (4): Degradation kinetics of DM, OM and CP for experimental concentrate feed mixtures.

	CFM	CFMU[1]	CFMU[2]	CFMU[3]	CFMH[1]	CFMH[2]	CFMH[3]	CFMI[1]	CFMI[2]	CFMI[3]	CFMB[1]	CFMB[2]	CFMB[3]	SE±
Experienced concentrate feed mixtures														
DM														
a	28.27[a]	25.52	24.73	23.52	27.32	26.15	25.28	25.82	25.26	24.52	28.13	27.32	26.17	1.07
b	55.28[a]	53.82[ab]	51.42[b]	48.64[b]	54.42[a]	53.48[a]	50.54[b]	54.65[a]	53.68[ab]	51.52[b]	55.13[a]	54.62[ab]	52.43[b]	1.36
c	0.045	0.042	0.038	0.035	0.041	0.038	0.037	0.040	0.037	0.036	0.040	0.038	0.037	0.004
EDDM	55.82[a]	51.44[b]	47.00[bc]	44.02[c]	52.78[b]	50.00[b]	47.61[bc]	50.83[b]	48.25[bc]	46.00[bc]	53.52[ab]	51.21[b]	49.61[bc]	6.58
OM														
a	26.48[a]	24.36[ab]	22.17[b]	20.58[b]	25.28[a]	24.12[ab]	23.36[ab]	24.58[ab]	23.42[ab]	22.61[b]	25.72[a]	24.42[ab]	24.12[ab]	0.88
b	56.62[a]	54.67[ab]	52.53[b]	49.82[b]	55.52[a]	54.83[ab]	51.88[b]	55.76[a]	54.73[ab]	52.72[b]	56.87[a]	55.74[a]	53.80[ab]	0.67
c	0.052	0.048	0.042	0.039	0.051	0.049	0.047	0.050	0.048	0.047	0.052	0.050	0.049	0.006
EDDM	56.90[a]	52.21[b]	47.88[c]	42.61[d]	54.79[ab]	52.37[b]	49.66[bc]	53.56[ab]	50.86[bc]	47.88[c]	55.84[a]	52.94[b]	51.45[b]	7.62
CP														
a	23.42[a]	22.62[a]	22.23[a]	21.75[b]	23.18[a]	22.92[a]	22.34[a]	22.86[ab]	22.42[ab]	22.15[ab]	23.28[a]	23.12[a]	22.76[ab]	0.53
b	64.46[a]	60.82[ab]	58.33[b]	56.64[b]	62.18[a]	60.18[ab]	59.72[a]	63.82[a]	61.74[ab]	60.32[ab]	65.62[a]	64.53[a]	62.43[a]	0.65
c	0.054	0.051	0.046	0.042	0.053	0.052	0.050	0.051	0.049	0.047	0.054	0.053	0.051	0.005
EDDM	53.86[a]	50.72[b]	45.80[c]	44.86[c]	53.67[a]	52.17[a]	50.58[b]	51.84[ab]	49.71[b]	47.93[c]	54.26[a]	52.78[a]	51.24[ab]	1.43

a,b and c: means in the same row with different superscripts are significantly different (P<0.05).

Also, washing loss fraction (a) degradable fraction (b) rate of degradation (c) and effective degradability (ED) of DM and OM were higher (P<0.05) for both chemical and biological treatments (with 50 and 75% of JMI) as compared with untreated.. Lower soluble fraction (%) and rate of degradation were noticed with untreated JM ration for DM and OM degradation compared to the control and other experimental ration with treated JCM. Meanwhile, higher values were obtained for CFMs containing biologically (JMB) and heated treated (JMH) with (25% & 50% of JMB JMH levels) as compared with untreated groups. However, there were no significant differences between untreated and different treatments with 25% level concerning the DMD and OMD values. The treatment with bacteria slightly increased DMD and OMD than treatment with heat treatment. The decrease of degradability of CFMs containing untreated JMU may be due to the negative effect of trypsin inhibitor and lectin on ruminal microorganisms. Ahmed and Adam (1979) , Panigrahi *et al* ., (1984) and Karmen *et al* ., (2006) concluded that trypsin inhibitor content of JCM as well as other anti-nutritional compounds affect digestibility. The digestibility of CP for CFMs contained untreated JMU was lower than digestibility of CP for CFMs contained treated JM as a result to the high content of trypsin inhibitor on JMU. On the other hand, no significant differences were detected among rations on the final value obtained for EDCP, between treated JM (biological and chemical treatments) and untreated JM which may be as a result of the decrease in trypsin inhibitor activity and lectin (Table 4). The slightly higher degradability of CP with bacteria treatment than heat treatment, may be as a result to the over protection with heat treatment. These could be related to the less digestibility of them in the rumen and may also be due to the effect of anti-nutritive substances which can lead to less feed intake as well.

The major problem with utilizing JCM as a protein feed source has been stated for its toxicity, which is attributed to the presence of anti-nutritional compounds . However, the methods applied in this study had proved to have positive effect on its feeding value by lowering the level of anti- nutritional compounds .

The elimination of trypsin inhibitor and lectin compounds by either treatment with bacteria or chemical improved the utilization of JCM as a new protein source. However, further studies are needed for long run trials in order to define the metabolic compounds which could be found as residual in the end products (milk and meat) of animals fed such Jatropha meal JCM.

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التقييم الغذائى لكسب الجاتروفا المعامل حراريا أو كيماويا أو بيولوجيا
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استهدفت الدراسة التقييم الغذائى لكسب الجاتروفا غير المعامل أو المعامـل حراريـا أو كيماويـا
بالأيزوبروبيانول أو بيولوجيا بالبكتريا. وقد اشتمل التقييم تقدير معدل اختفاء المادة الجافة والمادة العـضوية
والبروتين الخام وذلك باستخدام تقنية *In situ* وذلك باستخدام زوج مـن العجـول الجاموسـى مـزودة
بفستيولات الكرش لقياس نشاط الكرش لتقدير معدل تحلل المادة الجافة والمادة العضوية والبروتين الخام فـى
الكرش. وتمت تغذية الحيوانات على قش أرز بمقدار ٣/١ مقررات الحيوان اليومية بينمـا أعطـى العلـف
المركز المختبر بمعدل ٣/٢ من هذة المقررات. كما تم تقدير anti-nutritive compounds فى كل مـن
كسب الجاتروفا غير المعامل أو المعامل حراريا أو كيماويا أو بيولوجيا.
وكانت العلائق المستخدمة كما يلى :
١-قش أرز + علف مركز (كنترول) ع٠٠
٢-قش أرز + علف مركز يتم استبدال كسب فول الصويا بمعدل ٥٠،٢٥، ٧٥% بواسطة كسب الجاتروفـا
غير المعامل للعلائق ع١،ع٢،ع٣ على التوالى.
٣-قش أرز + علف مركز يتم استبدال كسب فول الصويا بمعدل ٢٥ ،٥٠، ٧٥% بواسطة كسب الجاتروفا
المعامل حراريا للعلائق ع٤،ع٥،ع٦ على التوالى.
٤-قش أرز + علف مركز يتم استبدال كسب فول الصويا بمعدل ٢٥ ،٥٠، ٧٥% بواسطة كسب الجاتروفا
المعامل بالبكتريا للعلائق ع٧،ع٨،ع٩ على التوالى.
٥-قش أرز + علف مركز يتم استبدال كسب فول الـصويا بمعـدل ٢٥ ،٥٠، ٧٥% بواسـطة كـسب
الجاتروفا المعامل كيماويا للعلائق ع١٠،ع١١،ع١٢ على التوالى.
وقد أدت المعاملات المختلفة الى خفض تركيزات المواد المثبطة للتغذيـة الـى الحـدود الأمنـة
لاستخدامها فى علائق المجترات. وقد أدت المعاملة البيولوجية باستخدام البكتريا الى زيادة محتوى الكـسب
من البروتين الخام ، بينما أدت المعاملة الحرارية الى خفض البروتين الخام . وقـد أدت كل من المعاملـة
البيولوجية والكيماوية الى خفض نسبة الألياف الخام، بينما لم تؤثر المعاملة الحرارية على نـسبة الأليـاف
الخام. بالنسبة لمعدل تحلل المادة الجافة والعضوية لمخاليط العلف المركز فى الكرش كان أعلاها فى العليقة
الخالية من كسب الجاتروفا، بينما فيما يختص بالعلائق المحتوية على نسب المختلفة من كسب الجاتروفا فلقد
سجل مستوى ٢٥% أعلى معدل اختفاء لكل من المادة الجافة والعضوية مـع جميـع المعـاملات مقارنـة
بمستويات الأخرى. ولقد سجلت أعلى قيمة لمعدل اختفاء كل من المادة الجافة والعضوية المخاليط المحتوية
مع نسب استبدال ٢٥، ٥٠، ٧٥% من كسب الجاتروفا المعامل بيولوجيا مقارنة مع المخاليط المحتوية على
كسب جاتروفا معامل كيماويا أو حراريا. بينما سجلت المخاليط المحتوية على كـسب جاتروفـا المعامـل
حراريا بمستويات ٢٥، ٥٠، ٧٥% أقل قيم بالنسبة لمعدل اختفاء كل من المادة الجافة والعضوية. بالنسبة
لمعدل اختفاء البروتين الخام فقد سجل المخلوط المحتوى على كسب جاتروفا معامل بيولوجيا أعلـى قيمـة
مقارنة بقيم المعاملات الأخرى. ونستخلص من ذلك ان طريقة المعاملة البيولوجية هى أكثر الطرق كفاءة فى
تقليل تركيز وتأثير المواد المثبطة للتغذية مما يساعد على الاستفادة من هذا المنتج كمـصدر علفـى غيـر
تقليدى بعد إجراء مزيد من التجارب التطبيقيه على الأداء الإنتاجى ومتبقيات المواد الضارة فى اللحم واللبن.

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