INTERIN PROGRESS REPORT No. 3

January 1, 1991 - June 30, 1991

PROJECT AID-SCI 936-5542-12

USE OF PORO (*Erythrina* spp.) AND MADERO NEGRO (*Gliricidia sepium*) AS A PROTEIN SOURCE FOR DAIRY CATTLE

CATIE

Turrialba, Costa Rica

1. NUTRITIVE VALUE

1.1 Chemical composition and in vitro dry matter disapperance

In May, 1991 it was realized the second sampling (dry season) of the three species in study (Erythrina poeppigiana, E. berteroana and Gliricidia sepium) in the tree different ecological enviromment (Guapiles, Turrialba and Puriscal) (see Interin Report No. 1).

The samples were dried and ground for further analysis of crude protein, in vitro dry matter digestibility, cell wall content and composition. These results will be presented and statistically analyzed with the first sampling (wet season), in the next report.

Also, in the next period (July to December, 1991) all samples from the two periods will be analysed for tannins and coumarins (Gliricidia) and alkaloids (Erythrina), in sacco dry matter and nitrogen disappereance in the rumen (Kass and Rodríguez, 1989).

1.2 Alkaloids from Erythrina species

In the methodological development of alkaloids (isolation and quantification) some progress have been made by the jointly efforts from CATIE's Laboratory and University of Missouri. The consultation made by Drs. G. Rottinghaus and G.

Garner in abril played a key role on this research. In this occasion it was fed to fistulated milking goat Erythrina seeds for one week. Rumen liquor and milk samples were taken and froze dried for further alkaloids analysis. It was observed that beta-erythroidine, the main Erythrina species alkaloid is hydrogenized in the rumen to dihydroerythroidine and tetrahydroeythroidine and that they are also present in the milk. Dr. Rottinghaus took more Erythrina seeds to his laboratory at the University of Missouri for further studies.

The work on alkaloide quantification is still in progress but going slowly to problems related to standards as described by Dr. Rottinghaus in his latest letter (Annex 1).

In our laboratory, we already have a relatively simple method to analyse alkaloides in Erythrina leaves, but we still want to do more analysis in order to use it as routine (see annex 2).

2. FEEDING TRIALS

2.1 Gliricidia sepium and Erythrina poeppigiana as a protein supplement to dairy cows fed on Jaragua hay (Hyparrhenia rufa)

As was indicated in the Interin Report No. 2, this research has as objective to evaluate the effect of Erythrina poeppigiana and Gliricidia sepium foliages as protein supplements, as compared to urea, on the milk yield and composition for dairy cows fed on low quality hay.

This research was carried out on CATIE's experimental farm with the following climatic conditions:

Latitude: $9^{o}58$'N

Longitude: $83^{o}31$'

Altitude: 639 m

Annual average precipitation: 2600 mm

Annual average relative humidity: 90.4%

Annual average temperature: 22.1oC

Twelve pure and cross bred Criollo x Jersey multiparous cows, between 45 to 60 days post partum were distributed in a replicate change over 3 x 3 Latin Square, with four replications, three cow per square, and three periods, with 21 days each (14 days of adaptation to each experimental diet and 7 days of measurement of milk yield and composition).

The cows were housed individually, in order to record the exactly intakes of hay and supplements per cow per day.

All cows received hay as the basic diet, protein supplement (Erythrina, Gliricicia or urea) and energy supplements (molasses, rice bran) according to their requeriments to produce 6.5 kg milk/day with 4% fat. All diets were isocaloric and isonitrogenous (see tables 1 and 2).

The results showed in table 3 indicated that milk yields for cows supplemented with Erythrina or Gliricidia foliage were statistically the same and as were planned in the diets (table 2); but urea supplementation gave lower ($P<0.05$) milk yields than the tree foliages and inferior to the calculated diet (table 2).

In relation to the milk composition all diets behaved alike (table 3).

The Parcial Budget Analysis, also indicated that the tree foliages supplemented diets gave higher net income than the urea supplemented diet (table 7).

In order to stablish the effect of the protein supplements on the hay degradability and the degradability of the supplements in the rumen, it was carried out a experiment with rumen fistulated steers fed at maintenance level.

Three animals were utilized in a 3 x 3 (3 diets and 3 periods) latin square design.

The diets were the same as in the experiment with dairy cows (table 2) and the periods lasted for 21 days: 14 days adaptation to experimental diets and 7 day of mesurements. The methodology utilized was the same described by Orskov and Mc Donald (1979), with incubation times: 0, 1.5, 3, 6, 12, 24, 36, 42, 72, 96 hours.

Addicionally, it was measured the rate of passage of the diets, utilizing the methodology described by Van Soest, Uden and Wrick (1983), in order to calculate the real degradability (Orskov and Mc Donald, 1979).

Also, pH, ammonia nitrogen and volatile fatty acids from rumen liquor, were determined at 0,2 4, 8 and 12 hours after feeding the supplements (Kass and Rodriguez, 1989).

The different protein supplements had no affect on the rate of passage of the diet through the gastrointestinal tract. Also, diets ingredients degradability in the rumen had the same values in all diets estudied (tables 4 and 5).

However, volatile fatty acid production was higher for the tree foliage diets, and ammonia nitrogen higher for the urea diet. These results could be responsible for the difference on milk production, and loss of weight of the cows with urea supplementation.

From the results obtained in this research it can be cocluded that:

- The NRC tables is reliable to calculate nutrients requeriments for estabulated dairy cows in the humid tropic conditions.

- *Erythrina poeppigiana* and *Gliricidia sepium* have the same feed value for dairy cows when supplementing low quality hay diets.

- The net income for tree foliage supplementation is superior to urea supplementation, for milk production on low quality hay diets.

2.2 Effect of four levels of rice bran on milk yield and quality produced by grazing cows supplemented with *Erythrina poeppigiana*

In tropical forages, protein and energy concentration are not enough to maintain milk production (Villegas, 1979). The utilization of supplements is a common management practice in most milk production systems in this region. In the other hand, traditional supplements (oil seed cakes, animal by products, etc.) are expensive and competitive to monogastric feeding systems.

The main objetive of this study was to evaluate from a bioeconomical point of view the effect of four levels of energy supplementation to dairy cows fed on Erythrina plus pasture diet.

The experiment was conducted under tropical rain forest condition at Turrialba, as discuted in the previous feeding trial.

Twelve pure and cross bred multiparous cows (Criollo x Jersey) were distributed in a replicated change over 4 x 4 Latin Square, with four replications, four cows per square and four periods.

The cows were maintained in a 5.7 ha pasture composed mainly by Cynodon nlemfluensis (8% crude protein and 54% IVDMD) divided in 9 plots (table 8). They received daily, in the milking parlor, o.4% of Erythrina per 100 kg live weight, one kg of molasses and rice bran according to the treatments: 0% LW, 0.2% LW, 0.4% LW and 0.6% LW (table 8).

The cows entered the experiment at aproximately 60 days post partum. Each period had 14 days for adaptation on the respective experimental diet and 7 days measurement of dry matter intake, milk production and quality. The amount of pasture intake was determined utilizing the indicator method as described by Iturbide (1967). Erythrina intake was calculated by the difference between foliage offered and refused (orts).

At the end of the study the diets were analysed economicaly by the method developed by Dillon and Hardaker (1980).

The results indicated that the supplementation with rice bran had a significant effect ($P< 0.01$) on milk production of the cows fed on pasture plus Erythrina diet (Figure 3 and Table 9). This effect can be due to the increase on by-pass energy and protein or increased microbial production, which could be used in the synthesis of milk protein and lactose (Figures 4 and 5, table 10), (Sporndly, 1989; Elliot et al, 1978).

The greatest increase in milk yield was observed with the supplementation of 0.2% LW of rice bran, probably due to better equilibrium between animals requeriments and nutrients intake (table 10). The feed quality of pasture and Erythrina (table 8) could be undestimated because of animal selection (pasture availability 63 $\pm$ 10 kg DM/animal/day; Erythrina 0.4% LW $\pm$ 10%).

Also, the experimental animal did not loose weight in this study (table 11), indicating that the requeriment for milk production was more than covered by all diets (table 10). It has to be noted that the requeriments did not include the expenditure of nutrients for grazing, walking, etc.

Moreover, the supplementation with 0.2% LW of rice bran required less crude protein and energy to produce 1 kg of milk (table 12), but increasing rice bran supplement above this level lowered the efficieny of utilization of the energy supplement to the level of no energy supplementation. This could be explained by the lower energy concentration of the forage diet as campared to the supplemented diets, and more energy expenditure to "harvest" the required feed (table 13 and 14; figures 4 and 5).

The only significant effect of rice bran supplementation on milk composition of cows fed on pasture plus Erythrina diet was for milk protein (table 9). The depression of milk fat with concentrate intake reported by several authors in the literature (Armstrong and Blaxter, 1965) was not observed in this research. Probably, intake of rice bran as compared to the other ingredients in the diet was low (figure 9) and insufficient to depress cellulotic activity in the rumen (Prieto and Sutherland, 1977).

Also, total milk solids were not affected by the treatments (table 9). The same results were obtained when cows on diets of Erythrina plus pasture were supplemented with molasses (Abarca, 1989).

In the other hand, milk protein content was linearly correlated to level of rice bran in the diet (table 9 and figure 10). Emery (1978) showed that an increase in energy intake also increases the content of milk protein in 0.015 unities/Mcal NE. Also, efficiency of milk protein production is greater for the supplemented diets than with forage (Erythrina plus pasture) diet (table 14). Therefore, Sporndky (1989) reported a high correlation betwen energy concentration in the diet and protein content in milk, as it was observed in this work (figures 11 and 12).

The amount of protein intake was also another factor that could affect the milk protein content in this research (figure 13). This effect was observed by Alagon (1990) when sugar cane was supplemented with fish meal and soybean meal as compared to Erythrina foliage and urea. Contraryly, Tobon (1989) did not find any increase on milk protein content with increasing levels of Erythrina foliage to cows grazing african star grass, perhaps due to soluble nature of the Erythrina poeppigiana crude protein (Espinoza, 1984).

The effect of rice bran supplementation on the pasture dry matter intake and total dry matter intake are shown on table 15. It can be noted that the level of supplementation (0.2, 0.4 and 0.6% LW) had the same effect, lowering pasture DM intake as compared to diet mith no supplementation; however o.4 and 0.6% LW rice bran supplementation affected more the total DM intake.

As can be observed on figure 14, the rice bran supplementation also improved Erythrina foliage dry matter intake, possibly due to increased rate of passage of diet or degradability in the rumen.

The Partial Budget Analysis presented in table 16 shows that both forage and 0.2% LW of rice bran supplemented diets had the highest net income as compared to 0.4 and 0.6% LW of rice bran supplementation.

In conclusion, 0.2% LW of rice bran supplementation to dairy cows in pasture and supplemented with 0.4% LW of Erythrina *poeppigiana* foliage improves milk production and quality, and has economical advantage as compared to higher levels of supplementation.

In order to elucidate the effect of rice bran supplementation on the rumen degradability of *Erythrina* when used as supplement to grass diets, it has been planned a trial utilizaing fistulated steers. All animals will receive *Pennisetum purpureum ad libitum*, *Erythrina poeppigiana* at the level of 0.4% LW and four levels of rice bran: 0, 0.2, 0.4 and 0.6% LW, in a 4 x 4 Latin Square design with 14 days of adaptation to experimental diet and 7 days meassurements of: dry matter degradability of *Erythrina*, rice bran and grass; rate of passage of diets; pH, ammomia nitrogen and volatile fatly acid in in the rumen liquor.

The methodology to be used will be the same as described in experiment 2.

2.3 Effect of energy supplement sources on milk yield and quality produced by grazing cows supplemented with *Erythrina poeppigiana*

In most tropical ecosystems, nutrition is the major limitant to animal productivity, being energy intake the main constraint. When, production systems are intensified, protein adquire importance as limiting nutrient.

The microbial population in the rumen, has the first opportunity to utilized the nutrients from the diet consumed by the animal. The microrganisms get the energy necesary to reproduce and growth, by the degradation of feed carbohydrates, producing as fermentation by-products volatile fatty acids, that are utilized as energy by the host animal (Smith, 1979).

Also, the degradation of nitrogenous compounds produces ammonia, which is converted to microbial protein, and digested and abserbed in the animal small intestine (Sattler and Roffler, 1975).

Issacson and Owens (1971) demostrated that the ammount of ammonia trapped as microbial protein, depends on the mumber and velocity of bacterial biomass growth. Then, the nitrogen fixation is proportional to the amount of available energy, or ammount of fermented feed.

Erythrina especies as Gliricidia sepium, have very soluble fermentable nitrogen, and if these feeds are not supplemented simultaneously with fast fermentable carbohydrates, the nitrogen will be lost for microbial growth, being absorbed by the rumen and lost in the urine.

The supplementation of Erythrina foliage with molasses (very high solubility) showed no improvement on milk yield when used at levels of 1.5 or 3 kg per animal day, when cows were grazing african star grass (Cynodon nlemfuensis) (Abarca, 1989). Also, the supplementation to cows on pasture plus 0.4% LW of Erythrina, with 0.2, 0.4 and 0.6% LW of rice bran showed an increase on milk yield and Erythrina intake. But, bypass nutrients and increased rate of passage of diets could be the reason for the increased milk yield, not better utilization of Erythrina.

In view of the results obtained untill now, a final research will be carried to evaluate four energy sources as supplement to grazing dairy cows feeding 0.4% LW of Erythrina foliage.

The energy sources will be:

Molasses - sugar, low protein

Rice bran - starch, medium protein, by product

Banana - starch, low protein

Sorghum - starch, medium protein, cereal grain

Twelve crossbred (Criollo x Jersey) multiparous cows will be distributed in a replicated change over 4 x 4 Latin Square design, with four treatments and three replications. A 21 days adaptation and a 7 days collection period will be used.

Foliage, grass and energy supplements will be analysed for dry matter and crude protein content. The response variables to be measured are: forage and supplements intake, and milk yield and composition.

Partial budget techniques will be used to economically evaluate the different treatments studied as described before.

In order to elucidate the effect of energy supplements on the rumen degradabilided of Erythrina and pasture, four fistulated steers will be used, with the same management as in the dairy cows feeding trial. The methodology to measure degradability will be the same as described before in this report.

3. CONSULTANCY

Between April 6 to 13 we got the visit of Drs. G. Rottinghaus (Chemist) and G. Garner (Nutricionist) from the University of Missouri. In this occasion, several technical and methodological were discused with CATIE's staff. Also, Dr. Rottinghaus and the Project chemist worked in the alkaloid methodology.

4. MEETINGS PARTICIPATION

Because of our work in this Project, we have been invited to participate in the Expert Consultation On Legume Tree and Other Fodder Trees As Protein Souces for Livestock, organized by FAO and to be held at MARDI, Kuala Lumpur, Malaysia, 14-18 october 1991. In this meeting we are going to present the results obtained in this project.

LITERATURE CITED

Abarca, S. 1989. Efecto de la suplementación con poró (Erythrina poeppigiana) y melaza sobre la producción de leche en vacas pastoreando estrella africana (Cynodon nlemfuensis). M.S. Thesis. CATIE, Turrialba, Costa Rica. 68 p.

Alagon, G. 1990. Comparación del poro (Erytrina poeppigiana) con otras fuentes nitrogenadas de diferente potencial de escape de la fermentación ruminal como suplemento de vacas lecheras alimentadas con caña de azúcar (Saccharum officinarum). M.S. Thesis, CATIE, Turrialba, Costa Rica. 145 p.

Dillon, J.I. and y B. Hardaker. 1980. Analisis del presupuesto parcial. In: La Investigación sobre Administración rural para el desarrollo del pequeño agricultor. Boletín de Servicios Agrícolas No., 41, FAO, Roma. p. 151-159.

Elliot, R., H.M. Ferreiro, A. Priego and T.R. Presto. 1978. Rice polishings as a supplement in sugar cane diets: the quantities of starch entering the proximal duodenum. Trop. An. Prod. 3:30-35.

Emery, R.S. 1978. Feeding for increased milk protein. J. Dairy Sci. 61:825-828.

Espinoza, J. 1984. Caracterización nutritiva de la fracción nitrogenada del follaje del madero negro (Gliricidia sepium) y poro (Erythrina peoppigiana). M.S. Thesis, UCR/CATIE, Turrialba, Costa Rica. 87 p.

Issacson, J. and F.N. Owens. 1971. 14C uptake by rumen microbes from urea and $NaHCO_3$. J. An. Sci. 33:287-290.

Iturbide, A.M. 1967. El óxido cromico como indicador externo para estimar producción fecal y consumo en las pruebas de digestibilidad. M.S. Thesis UCR/CATIE, Turrialba, Costa Rica. 137 p.

Kass, M. and G. Rodriguez. 1989. Manual de Evaluación Nutricional de pastos y forrajes. Area de Ganadería Tropical. CATIE, Turrialba, Costa Rica. 43 p.

Osrkov, E.R. and I. Mc Donald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92:499-503.

Priego, A. and T.M. Sutherland. 1977. El efecto del ácido propionico sobre el patron de fermentación ruminal. Prod. An. Trop. 2:192-197.

Sattler, L.D. and R.R. Roffler. 1975. Nitrogen requeriment and utilization in dairy cattle. J. Dairy Sci. 58:1219-1222.

Smith, N.E. 1979. Synthesis of microbial nitrogen compounds in the rumen and their subsequent digestion. J. An. Sci. 49:1604-1611.

Sporndly, E. 1989. Effects of diet on milk composition and yield of dairy cows with special emphasis on milk protein content. Swedich J. Agric. Res. 19:99-106.

Tobon, C. 1989. Efecto de la suplementación con tres niveles de follaje de poro (Erythrina poeppigiana) sobre la producción de leche de vacas en pastoreo. M.S. Thesis, CATIE, Turrialba, Costa Rica. 67 p.

Van Soest, P.J., P. Uden and K.F. Wrick. 1983. Critique and evaluation of markers for use in nutrition of humans and farm and laboratory animals. Nut. Rep. Int. 27:17-28.

Villegas, L.A. 1979. Suplementación con banano verde a vacas lecheras en pastoreo. M.S. Thesis, UCR/CATIE, Turrialba, Costa Rica. 65 p.

Table 1. *In vitro* dry matter digestibility and chemical composition of the diets ingredients.

	Hay	*E. poeppigiana*	*G. sepium*	Rice bran	Molasses
IVDMD%[1]	35	52	54	66	
Crude Protein%	4	26	27	12	6
NDF%[2]	83	59	53		
ADF%[3]	57	34	32		
Hemicellulose%	24	25	21		
Cellulose%	38	21	19		
NDF-N%[4]	53	53	47		
ADF-N%[5]	13	13	14		
ADF-Lignin%	19	13	12		
ME, Mcal[6]	1.11	1.95	2.01	3.38	2.76

1 IVDMD - *in vitro* dry matter digestibility
2 NDF - neutral detergent fiber
3 ADF - acid detergent fiber
4 NDF-N - nitrogen linkage to NDF
5 ADF-N - nitrogen linkage to ADF
6 Metabolizable energy
Molasses from NRC tables
Other ingredients calculates as:

$$DE = \frac{TDN\%}{100} \times 4.409$$

$$ME = -0.45 + 1.01\ DE$$

Table 2. Composition of experimental diets utilized in feeding trial 2.1.

Ingredients	% in diet	Dry matter intake kg/day	Crude Protein intake g/day	Metabolizable energy intake Mcal/day
		DIET 1		
Hay	3.940	44	158	4.37
E. poeppigiana	1.630	18	423	3.08
Rice bran	1.940	19	233	6.56
Molasses	1.710	19	103	4.72
TOTAL	9.220	100	917	18.73
		DIET 2		
Hay	3.940	44	158	4.37
G.sepium	1.630	18	440	3.18
Rice bran	1.940	19	233	6.56
Molasses	1.710	19	103	4.72
TOTAL	9.220	100	934	18.83
		DIET 3		
Hay	4.570	50	183	5.07
Urea	0.115	1	316	0.00
Rice bran	2.280	28	274	7.71
Molasses	2.150	21	129	5.93
TOTAL	9.110	100	902	18.77

1 Diets calculated to meet the requeriments of cows with intake of 3 kg DM/100 kg Live weight and producing 6.5 kg of milk with 4% fat.

Table 3. Effect of protein supplement on milk production and milk composition of cows fed hay diet.

	DIETS		
	Erythrina	Gliricidia	Urea
Milk production (kg/day)	7.3a	7.4a	6.7b
Corrected milk production (kg/day)[1]	6.7a	6.8a	6.2b
Fat %	3.4a	3.4a	3.5a
(g/day)	250a	250a	230b
Protein %	2.9a	2.9a	2.8a
g/day	210a	210a	180b
Total solids %	11.9a	11.8a	11.9a
g/day	870a	880a	790b

a, b Means with different superscripts in the same row are different (P< 0.05).

1 Corrected to 4% Fat

Table 4. Rate of passage, retention time and transit time of hay diets with different protein supplements.

Diets	Rate of passage (%)		Retention time (hours)		Transit time (days)
	Rumen	Lower tract	Rumen	Lower tract	
Erythrina	2.7	6.7	37	15	6.9
Gliricidia	2.7	7.0	36	14	7.0
Urea	2.6	7.5	38	13	7.1

Table 5. *In situ* dry matter degradability of diets ingredients when different protein sources were supplemented to a basic hay diet.

	DIETS							
		Erythrina			Gliricidia		Urea	
	Erythrina	Hay	rice bran	Gliricidia	Hay	rice bran	Hay	rice bran
Initial (%)	30	10	31	28	10	33	9	31
Potential (%)	65	67	82	75	68	84	65	83
Real (%)[2]	37	31	41	37	31	43	30	42
Rate (hours)	0.15	0.02	0.09	0.11	0.02	0.09	0.03	0.09
T/2 (hours)[3]	5	39	7	6	40	8	41	7

1 Calculated from Y = a + b (1-e-CT) where:
Y = degradability at incubation time t
a = initial degradability t = o
a + b = potential degradability, time not limited
c = rate of degradation
t = incubation time
Real degradability = A+ (B x C/C + K)
Where K = rate of passage

3 T 1/2 = half life in the rumen = Ln 2/c.

Table 6. Rumen fermentation parameters of hay diets supplemented with different protein sources.

Parameters	DIETS		
	Erythrina	Gliricidia	Urea
pH[1]	6.6a	6.5a	6.4a
NH_3-N, mg/100 ml[2]	19.2b	21.3b	31.8a
Total VFA, umol/ml[3]	98.8a	110.7a	83.9b
Acetic Acid			
umol/ml	71.0a	78.9a	58.7b
%	72a	71a	70a
Proponic Acid,			
umol/ml	14.8a	16.9a	10.4a
%	15a	15a	12a
Butyric Acid,			
umol/ml	13.1a	14.9a	14.8a
%	13a	14a	18a

a, b Means with different superscripts in the same row are different ($P < 0.05$).
1 pH from rumen liquor
2 Ammonia nitrogen in rumen liquor
3 Total volatile fatty acids.

Table 7. Partial Budget Analysis for the hay diets supplemented with different protein sources.

Income	Protein supplements		
	Erythrina	Gliricidia	Urea
Milk production, kg	7.30	7.40	6.70
Cost per kg (US$)	0.30	0.30	0.30
Gross income (US$)	2.19	2.22	2.01
Variable costs (US$/kg)			
Hay	.25	.25	.30
Rice bran	.40	.40	.45
Molasses	.07	.07	.08
Erythrina	.10	-	-
Gliricidia	-	.10	-
Urea	-	-	.03
Total	.82	.82	.86
Net income			
US$/cow/day	1.37	1.40	1.15
% total income	62.55	63.06	57.21

Table 8. Composition of feedstuffs fed to the cows on pasture and supplemented with Erythrina and four levels of rice bran.

Feedstuff	Dry matter %	Crude protein %	IVDMD[1] %	Ether extract %
Rice bran	91	13	65	14
Erythrina	20	27	54	
Pasture	32	8	54	
Molasses	75	6		

1 *In vitro* dry matter digestibility.

Table 9. Effect of level of rice bran in the milk yield and composition of cows fed on pasture plus Erythrina diet.

Level of rice bran kg/100 kgLW[1]	Milk production kg/cow/day	Fat %	Protein %	Total solids %
0	8.8	3.5	3.1	12.1
0.2	9.6	3.6	3.1	12.2
0.4	9.9	3.5	3.2	12.1
0.6	10.5	3.4	3.2	12.1

1 LW = live weight.

Table 10. Nutrient requeriment and nutrient intake for each level of rice bran supplementation to pasture plus Erythrina diets.

Level of rice kg/100 kg LW	Milk production kg/cow/day	Crude protein g/day		Digestible energy Mcal/day	
		Required	Intake	Required	Intake
0	8.8	991	1037	23.4	24.4
.2	9.6	1061	1071	24.4	24.1
.4	9.9	1079	1159	24.6	26.7
.6	10.5	1136	1262	25.6	28.7

Table 11. Effect of levels of rice bran supplementation on the daily gain of cows on pasture plus Erythrina diets.

Level of rice bran kg/100 kg LW	Average daily gain g/animal
0	128
.2	285
.4	57
.6	48

Table 12. Efficiency of nutrients utilization for milk production from cows fed on pasture plus Erythrina diet and different levels of rice bran supplementation.

Level rice bran kg/100 kg LW	Crude protein g/kg milk	Digestible energy Mcal/kg milk
0	118	2.75
.2	111	2.49
.4	117	2.70
.6	120	2.74

Table 13. Energy concentration of pasture plus Erythrina diets supplemented with different levels of rice bran.

Level rice bran kg/100 kg LW	Mcal of DE/kg DM
0	2.41
.2	2.49
.4	2.56
.6	2.62

Table 14. Relationships between levels of rice bran and efficiency of energy utilization to produce milk protein of cows on pasture plus Erythrina diets supplemented with different levels of rice bran.

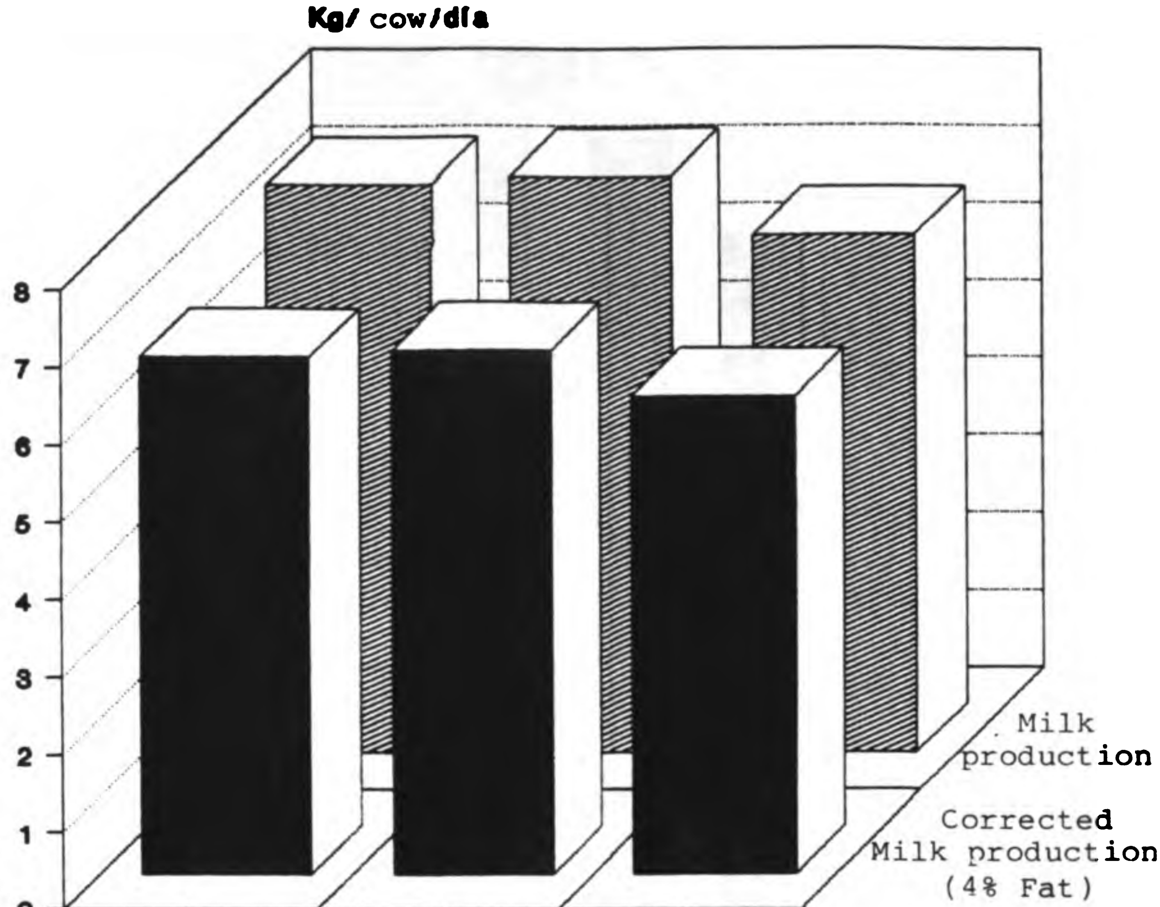
Levels of rice bran % LW	DE from Rice bran %/total energy	Efficiency of milk protein production g Prot./Mcal DE
0	0	11.1
.2	9.5	12.4
.4	17.0	11.7
.6	23.8	11.6

Table 15. Effect of rice bran supplementation levels in the pasture, Erythrina and total intake of cows fed on pasture plus Erythrina diets.

Level of rice bran kg/100 kg LW	Average daily intake (kg/100 kg LW)		
	Pasture	Erythrina	Total
0	2.58	0.40	2.98
.2	2.16	0.46	2.82
.4	2.24	0.46	3.09
.6	2.15	0.49	3.24

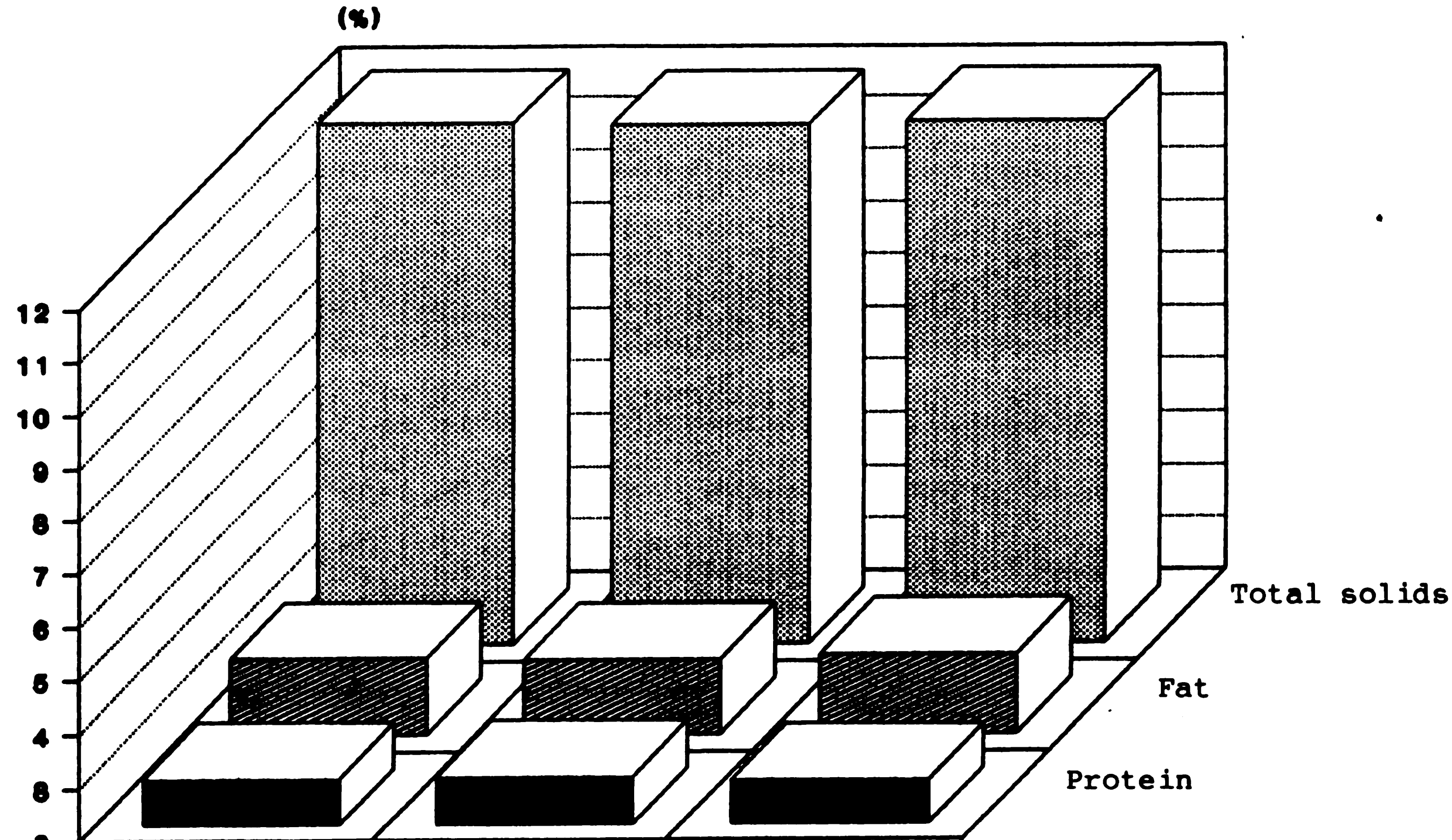
Table 16. Partial budget analysis for the pasture plus Erythrina diets supplemented with different levels of rice bran.

Income	Levels of rice bran (kg/100 kg LW)			
	0	0.2	0.4	0.6
Milk production	8.83	9.66	9.88	10.54
Cost per kg (US$)	0.30	0.30	0.30	0.30
Gross income	2.65	2.90	2.96	3.16
Variable costs (US$)				
Molasses	0.05	0.05	0.05	0.05
Rice bran	-	0.11	0.22	0.33
Erythrina	0.11	0.12	0.12	0.13
Pasture	0.14	0.12	0.12	0.12
Total	0.30	0.40	0.51	0.63
Net income				
US$/cow/day	2.35	2.50	2.45	2.53
% total income	88.68	86.21	82.77	80.06



	Erythrina	Gliricidia	Urea
Milk prod.	**7.33** a	**7.44** a	**6.68** b
Milk 4% Fat	**6.68** a	**6.76** a	**6.16** b

Figure 1. Total milk production and corrected milk production (4% Fat) from cows fed on Jaragua hay as affected by protein supplement sources.



	Erythrina	Gliricidia	Urea
Solids	11.85 a	11.81 a	11.87 a
Fat	3.42 a	3.33 a	3.47 a
Protein	2.86 a	2.89 a	2.82 a

Figure 2. Milk composition from cows fed Jaragua hay as affected by the protein supplement sources.

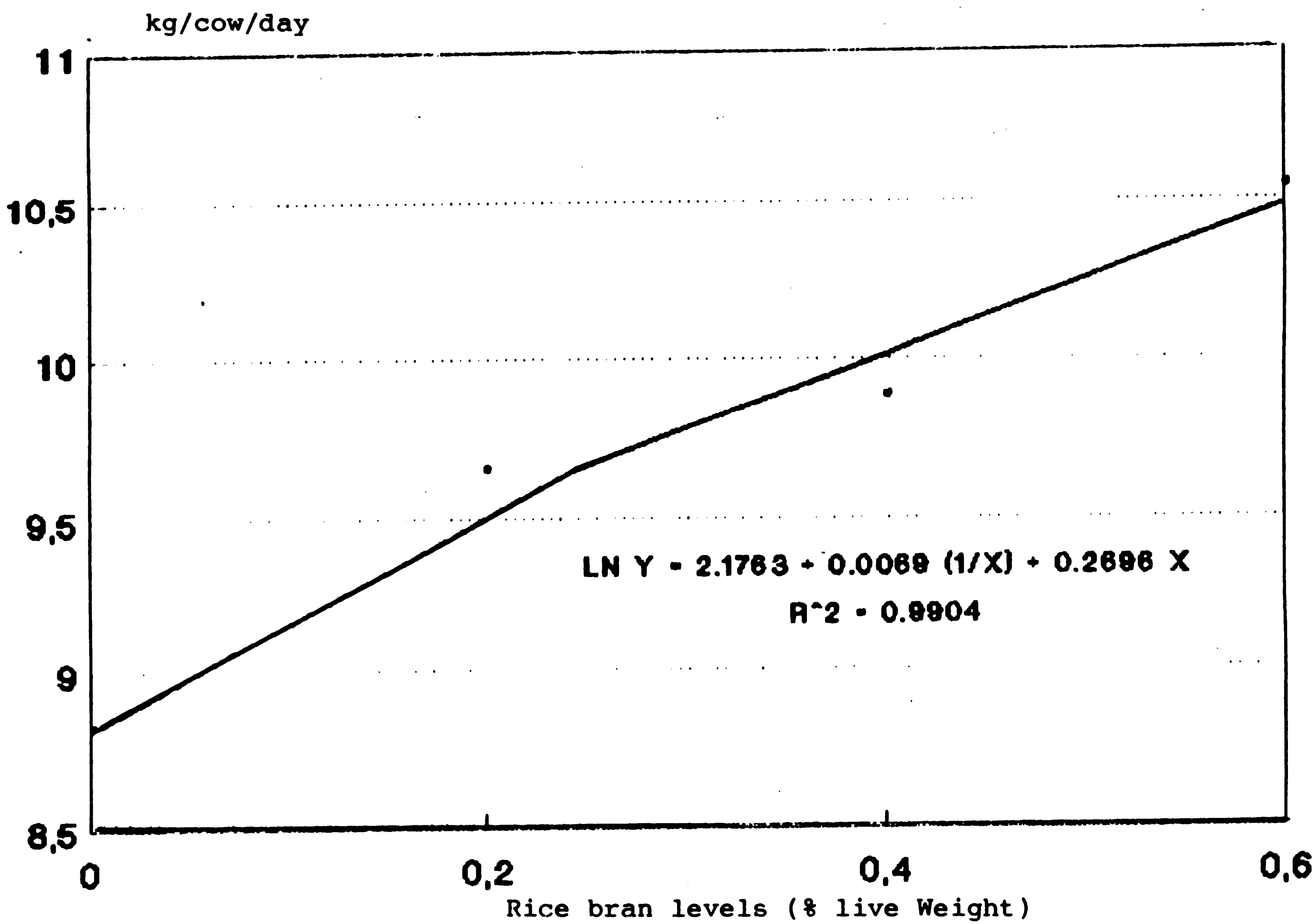


Figure 3. Milk production from cows fed on pasture plus Erythrina as affected by the level of rice bran supplementation.

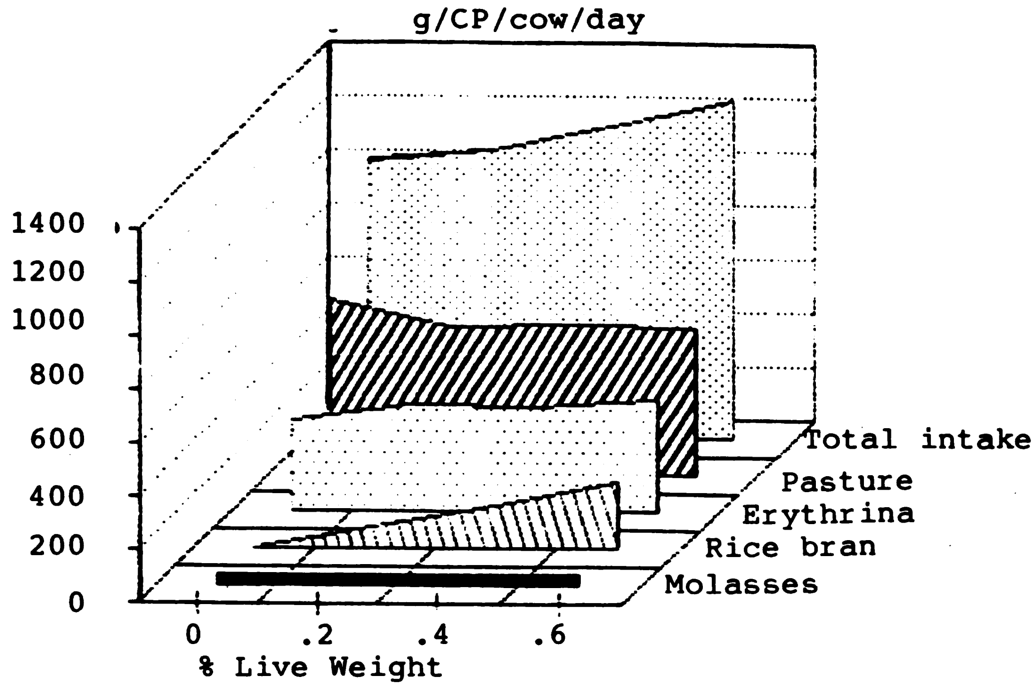


Figure 4. Crude protein intake by cows fed on pasture plus Erythrina supplemented with different levels of rice bran.

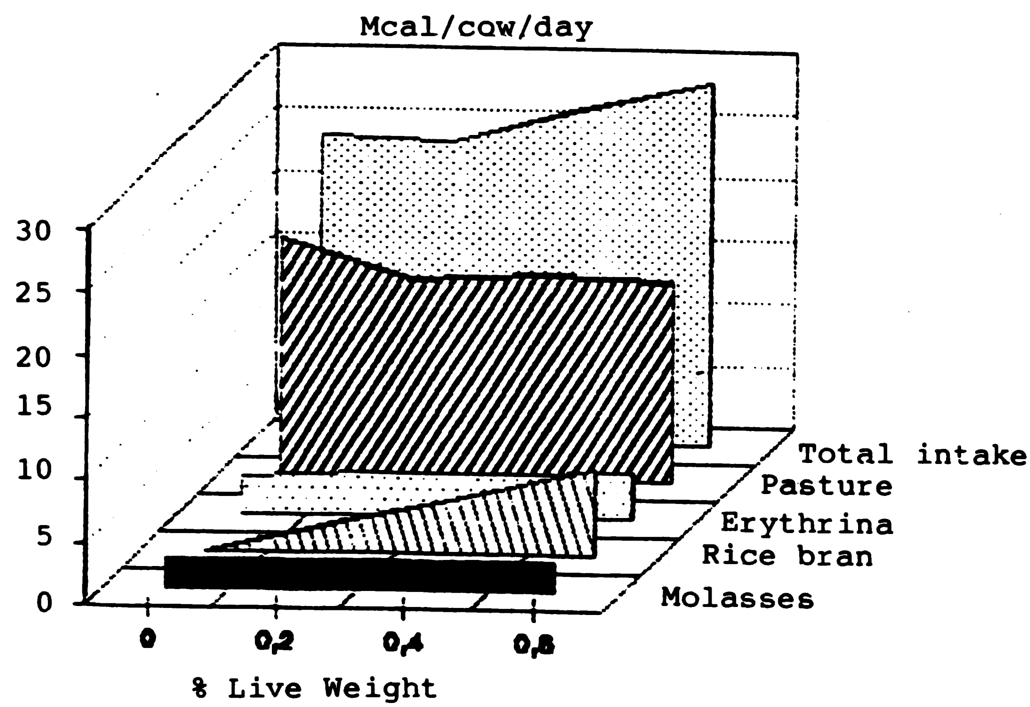


Figure 5. Digestible energy intake by cows fed on pasture + Erythrina supplemented with different levels of rice bran.

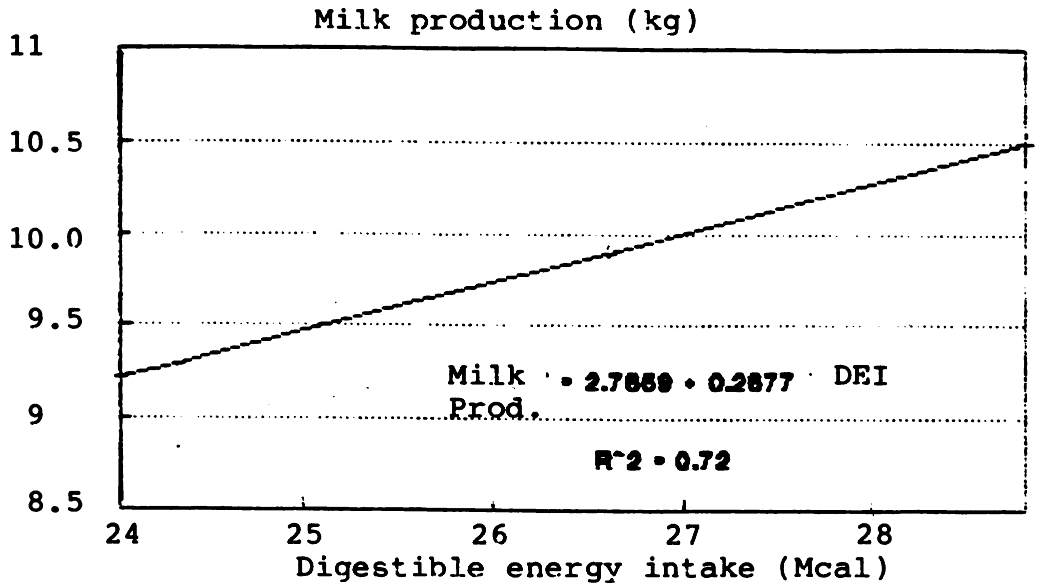


Figure 6. Efect of digestible energy intake on the milk production of cows fed on pasture plus Erythrina and supplemented with different levels of rice bran.

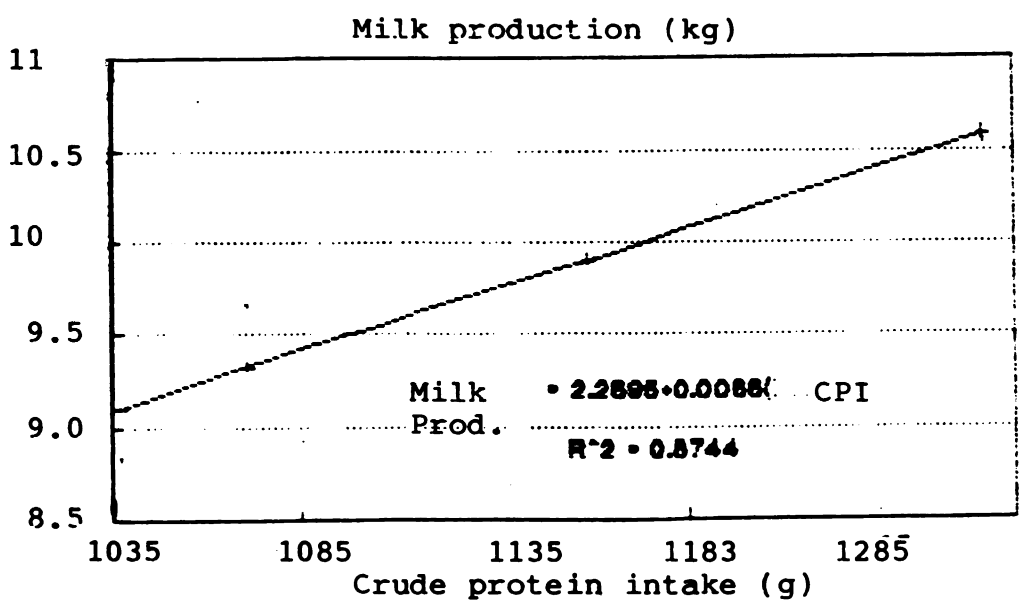


Figure 7. Efect of crude protein intake on milk production of cows fed on pasture plus Erythrina supplemented with different levels of rice bran.

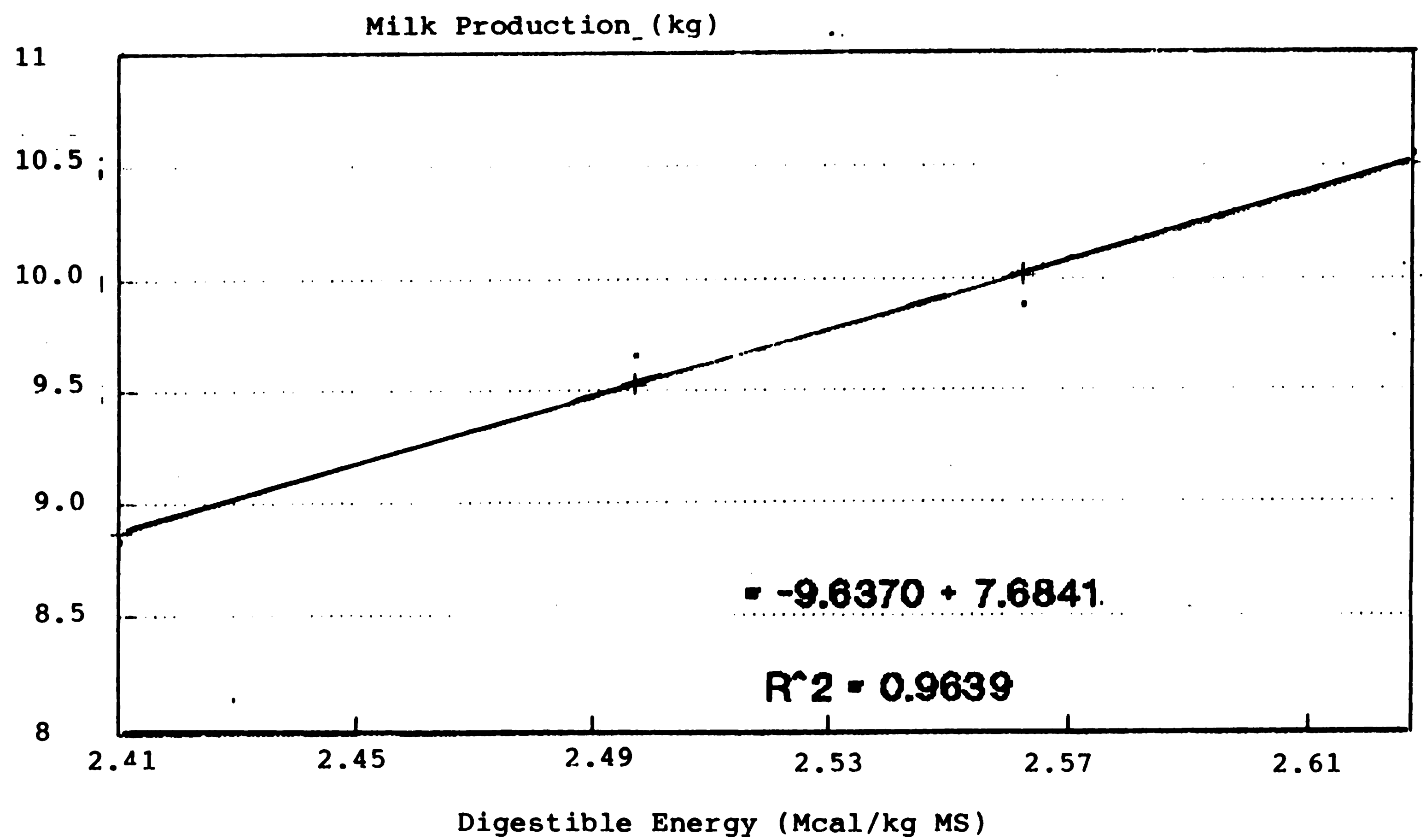


Figure 8. Efect of digestible energy concentration on the diet on the milk production of cows fed on pasture plus Erythrina and supplemented with different levels of rice bran.

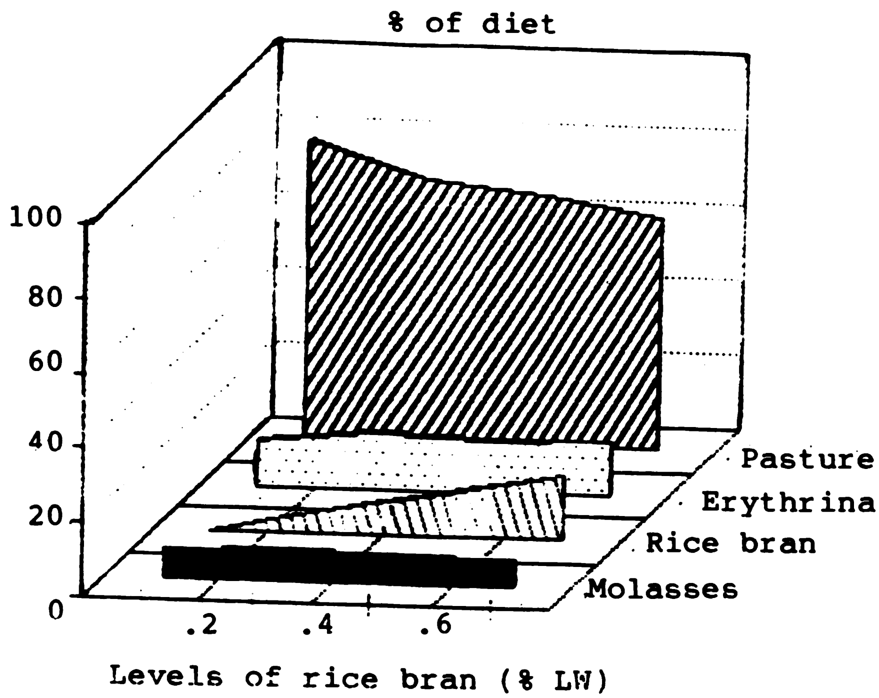


Figure 9. Intake of each diet ingredient as compared to total dry matter intake.

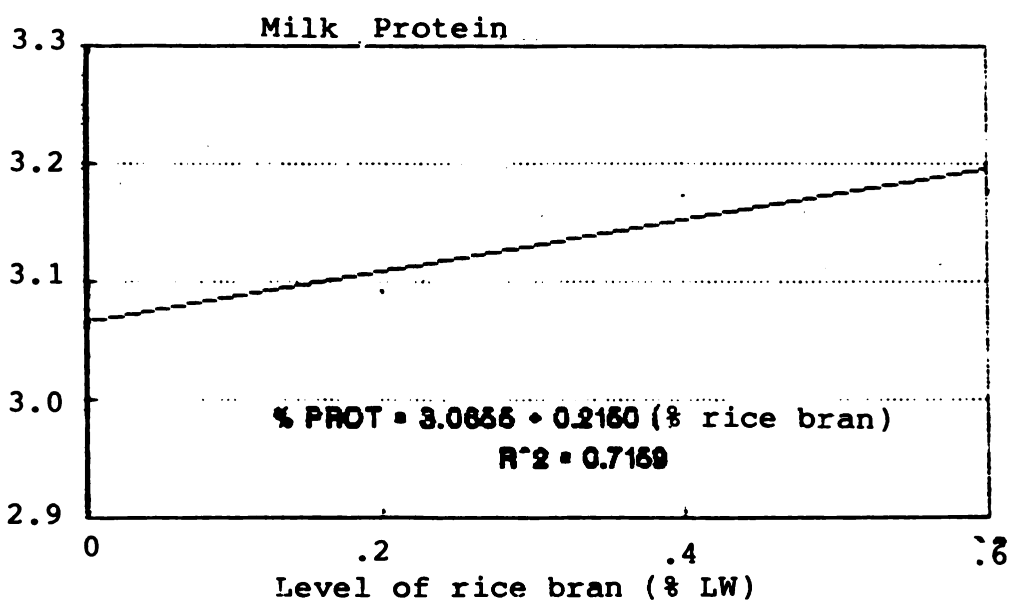


Figure 10. Efect of level of rice bran in the diet on the milk protein content of cows fed on pasture plus Erythrina.

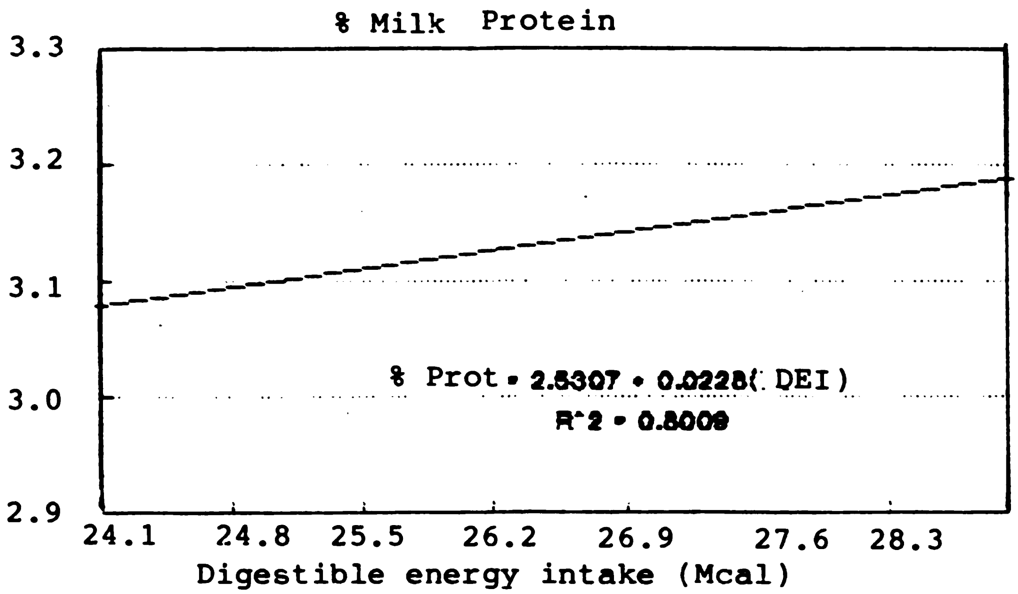


Figure 11. Effect of digestible energy intake on the milk protein content of cows fed on pasture plus Erythrina and supplemented with rice bran.

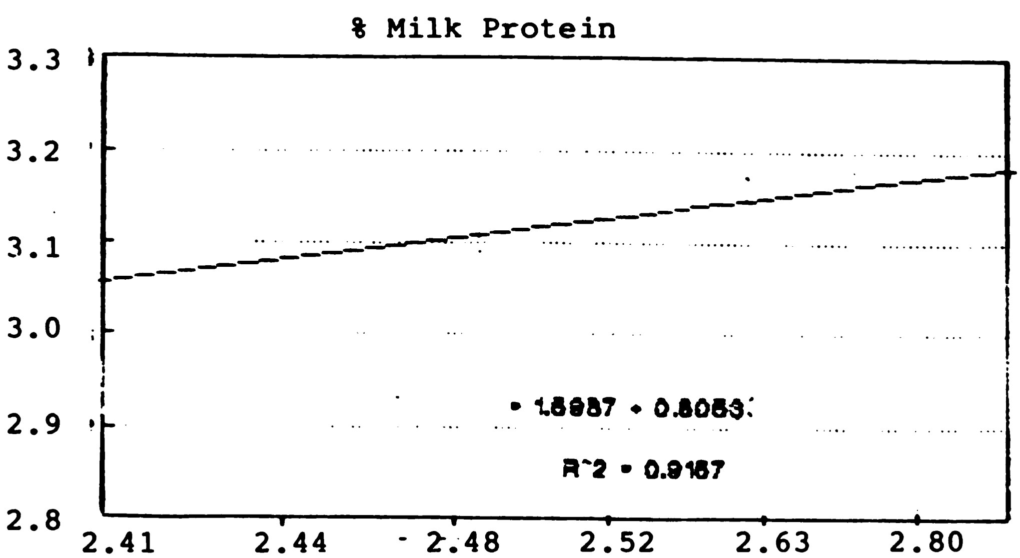


Figure 12. Effect of digestible energy concentration in the diet on the milk protein content of cows fed on pasture plus Erythrina and supplemented with different levels of rice bran.

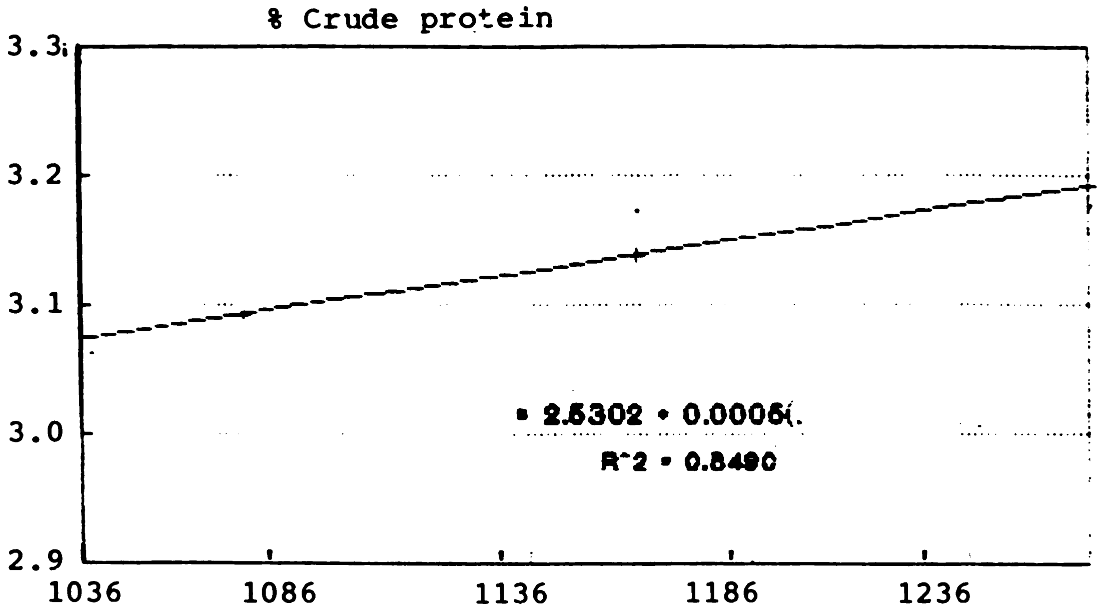


Figure 13. Effect of crude protein intake on the milk protein content of cows fed on pasture plus Erythrina and supplemented with rice bran.

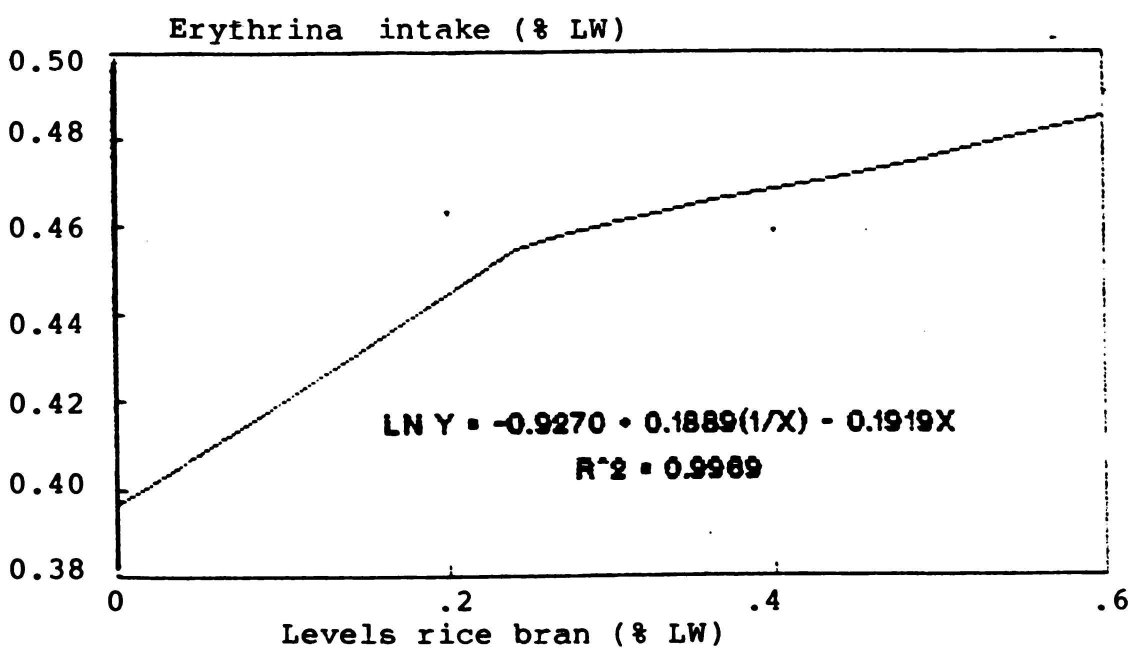


Figure 14. Effect of rice bran levels on the diet on the Erythrina intake of cows on pasture.

ANNEX

ANNEX 1

UNIVERSITY OF MISSOURI-COLUMBIA

College of Veterinary Medicine

Veterinary Medical Diagnostic Laboratory

P.O. Box 6023
Columbia, Missouri 65205
Telephone (314) 882-6811

August 19, 1991

Dr. Maria Kass
Nutritionist
CATIE
Turrialba
Costa Rica

Dear Maria:

Sorry about the delay, but I have been swamped with the wheat scab problem that is in the Midwest United States. We're running hundreds of samples of wheat for the mycotoxin, vomitoxin.

We were unable to find a qualified person to work for the summer, therefore we have not spent any of the money. I have hired a biochemistry student and a veterinary student who will start work at the end of August on the project. Both have experience in the laboratory and hopefully, this will allow us to get alot accomplished in a hurry. The money we have should get us almost through the Fall semester. I'm sure glad to get a couple of people that can help!

Sorry, we really do not have much to report. Linda and I have extracted almost all of the seed (except what we plan on feeding to a lactating goat) and have partially cleaned up the material. We have experimented with Sep-Pak cartridges for sample cleanup of milk and we're close, but not quite there yet, we do not have data on recoveries. This Fall the two new people will work on the separation of the alkaloids and the milk procedure. Hopefully, Dana (the vet student) can help us with the feeding trial. I have been trying to find a quick quantitative test for screening plant material for total alkaloids, But everything we find is from the 1950's and I don't consider the tests that quick and dirty.

I talked to Dr. Rhinehart and I'm not sure if we are going to get any alkaloid standards from his lab. That was work that was done along time ago and they can't find the compounds in his laboratory, though they are still trying to find them. It does not look promising.

I have mailed the Analtech catalog to Gerardo. We need the year or the editor of the book that has the chapter that he requested. There are about 150 books in that section of the library, all on phenolics, with about the same title. Diana spent an afternoon looking for it, but was unsuccessful with the information that we have. If you do not have any additional information, we will just have to go through them till we find it. Please, if you need supplies, just send me a list and I'll order as much as I can and send it down to you (eg. TLC plates).

Please keep me informed on the possibility of Gerardo coming to the University of Missouri for graduate school. I need to keep Dr. Manahan informed of our plans. Again sorry about our progress, but hopefully, we can accomplish alot this semester!

Sincerely,

George E. Rottinghaus

ANNEX 2

Alkaloid Analysis

1. Weight approximately 1 g of ground sample (1 mm) to a centrifuge tube.

2. Add 2 ml of 5% NaOH

3. Extract twice with 10 ml CHCl3

4. Shake 1 minute in vortex.

5. Filter with cotton to a 50 ml centrifuge tube.

6. Extract twice with 10 ml 10% HCl.

7. Separe the aqueous-acid phase and neutralize with 20% NaOH untill pH 12.

8. Filter in a 185 paper filter

9. Evaporate to dryness

10. Dilute the sample and read absorbance in a UVat 238 mm.