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FINAL REPORT

BIOLOGICAL UPGRADING OF PRETREATED COFFEE PULP

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Project No. 936-5544.21

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FINAL REPORT

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"BIOLOGICAL UPGRADING OF PRETREATED COFFEE PULP"

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Project Duration: 2 1/2 years.

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3 EXECUTIVE SUMMARY.

3.1 Purpose of the project:

To reduce the antiphysiological compounds (caffeine, polyphenols and potassium) present in coffee pulp by physical, chemical, or biological methods in order to utilize the treated material as ingredient in ruminant rations.

3.2 Principal findings:

A. The **ensilage** of coffee pulp reduced the contents of caffeine and polyphenols. Such reductions in caffeine, total polyphenols and condensed polyphenols ranged between 13-63%, 28-70% and 51-81% respectively. Weight losses (wet basis) during silage of fresh coffee pulp without additives increased in direct proportion with the silage time. Thus for 125 days of silage the weigh loss was 19.5 % and for 223 days was 26.9%. Mechanical pressing and addition of 5% of molasses (wet basis) before the silage improved the quality of the ensiled material.

B. Among **physical treatments** applied to fresh coffee pulp the sequence: screw pressing / hot water extraction / screw pressing presented the most promising results and produce an end product with attractive qualities for animal feeding (mainly ruminants). The reductions of anti-physiological compounds with such treatment were: caffeine 28%, total polyphenols 61%, condensed polyphenols 54%, potassium 20% and ash 25%.

C. Coffee pulp **bioconversion** with the fungus ***Penicillium crustosum*** produced a final product with promising characteristic for animal feeding. The reductions of anti-physiological compounds in the fermented product varied in the following ranges: caffeine 77-86% and total polyphenols 38-43%.

D. The **animal feeding** evaluations for the above materials were done using white New Zealand rabbits fed with four different diets, each containing 15% of one of the following materials: fresh coffee pulp, ensiled and pressed coffee pulp, ensiled and aqueous extracted coffee pulp and fungal fermented coffee pulp with ***Penicillium crustosum***, did not result in any significant difference ($P<0.05$) in weight gain with respect to control group. Statistical analysis showed significant differences ($P<0.05$) in relation to final weight, weight gain and feed consumption between sexes. Females animals presented the best performance.

3.3 Collaboration:

An active collaboration was established between The Institute of Central America and Panama (INCAP) and ICAITI aimed to the evaluation treated coffee pulp through animal feeding tests.

Collaboration with Israeli institutions (The Hebrew University of Jerusalem and The Volcani Center) was also established. The Hebrew University collaborated in preliminary microbiological screening tests and The Volcani Center performed digestibility tests of treated coffee pulp. The final reports of The Hebrew University and The Volcani center are included in Appendixes 6 and 7.

3.4 Expertise gained:

3.4.1. Technology:

Expertise in technology for the bioconversion of agricultural by-products through solid state fermentation and for the screening of fungi with certain characteristics against toxic compounds were gained. Simulation of cattle feeding test through utilization of pseudoruminants (rabbits) was done for the first time with these products.

4. RESEARCH OBJECTIVES.

4.1 Description of the problem:

The disposal of fresh coffee pulp represents a serious problem to the coffee factories (beneficios) and increase the processing costs. Unappropriated disposal methods of such residues cause severe pollution problems in water bodies, agricultural land or ravines.

Most of the important coffee beneficios in Central America are located near urban areas and the pollution created by disposal of coffee by-products (mainly grain washing water and fresh coffee pulp) are affecting the health of the population. Social pressure against such production centers have reached the point that local authorities have had to close temporally the factories with the consequent economic impact not only to coffee growers but to the national economy.

Previous research work on utilization of coffee pulp in animal feeding has demonstrated that this material has severe limitations when it is utilized in poultry or swine rations (Braham, 1979 and Jarquin, 1979) but ruminants were more tolerant (Cabezas et al, 1979). Poor palatability and the presence of substances with antiphysiological action such as 1) caffeine, 2) the free or monomeric phenols, chlorogenic, caffeic and tannic acids, 3) the polymeric phenols and 4) potassium seem to be responsible of the poor performance of diets containing coffee pulp (Bressani, 1979). However, subsequent research works have demonstrated that is technically feasible to reduce the contents of such undesirable compounds at such level that the final product present attractive

characteristics to be utilized in animal rations as sources of protein, fiber and energy (Gómez Brenes, 1979).

The present research was aimed to explore alternate routes for antiphysiological compounds reduction such as solid state fermentation with microbial strains with specific action against some undesirable compounds or to test combination of physical, chemical or biological methods such as pressing, alkaline or aqueous extractions, steam treatment, etc., which complement previous works.

ICAITI supported partially the costs of the research work and local institutions as The National Bureau of Coffee of Guatemala (ANACAFE) and The Institute of Central America and Panama (INCAP) collaborated directly in sample collection and animal feeding tests respectively. Israeli institutions collaborated in some aspects of the technical work.

5. METHODS AND RESULTS:

5.1 CAFFEINE REDUCTION IN COFFEE PULP THROUGH SILAGE:

5.1.1. ABSTRACT:

Silage tests to study reductions of antiphysiological compounds (caffeine and polyphenols) of fresh coffee pulp during the anaerobic fermentation were done. A concrete silo divided in compartments, with a total capacity of 9 tons of fresh material was utilized. The silage periods ranged between 99-224 days and the following materials were ensiled: 1) coffee pulp, 2) coffee pulp with sugar cane molasses, 3) coffee pulp with a mixture of molasses and ammonia and 4) screw pressed coffee pulp with molasses. Reductions in caffeine, total polyphenols and condensed polyphenols ranged between 13-63%, 28-70% and 51-81% respectively.

It was concluded that in the case of coffee pulp, silage presents and ideal method to preserve the material and partially reduce the contents of antiphysiological compounds .

5.2 REDUCTION OF ANTIPHYSIOLOGICAL COMPOUNDS OF COFFEE PULP THROUGH PHYSICAL TREATMENTS:

5.2.1 ABSTRACT:

Fresh coffee pulp was submitted to different physical and physico-chemical treatments in order to reduce the content of antiphysiological compounds (caffeine, polyphenols and potassium) interfering with the utilization of this by-product as ingredient in rations for animals (mainly ruminants). The following treatments

were utilized: screw pressing, steaming, aqueous extraction and extraction with a 4% ammonia solution.

With the best sequence: screw pressing/hot water extraction/screw pressing, the reductions of antiphysiological compounds from fresh coffee pulp were: caffeine 28%, total polyphenols 61%, condensed polyphenols 54%, potassium 20% and ash 25%.

The final product presented attractive qualities as feed for ruminants.

5.3 REDUCTION OF CAFFEINE OF COFFEE PULP THROUGH SOLID STATE FUNGAL FERMENTATION.

5.3.1 ABSTRACT:

Caffeine, polyphenols and potassium present in coffee pulp have proved their negative effects on animal metabolic performance when they are included in the diet.

The present research work was aimed to reduce caffeine and polyphenols in ensiled coffee pulp through solid state fermentation with the filamentous fungus strain *Penicillium crustosum*. The bioconverted product presented reductions of antiphysiological compounds in the following ranges, related to the fermentation time: caffeine 61-90%, polyphenols 13-61% and increments in ash 15-23%. The yield of fermented product was 89%. The reductions in dry and organic matter enzymatic digestibilities of coffee pulp were 22% and 28% respectively.

5.4 TREATED AND UNTREATED COFFEE PULP AS MAJOR COMPONENT IN PSEUDORUMINANT FEED.

5.4.1 ABSTRACT:

Animal feeding test with white New Zealand rabbit aimed to test two levels of inclusion of coffee pulp (15% and 40%) in the diet were done.
The following types of substitution products were tested: a) fresh coffee pulp, b) ensiled and pressed coffee pulp, c) ensiled and water extracted coffee pulp and e) fungal fermented coffee pulp with *Penicillium crustosum*.

It was concluded that the inclusion of 15 % of the above mentioned materials in different diets did not result in any significant difference ($P<0.05$) with respect to the control group.

5.5 EFFECT OF DIFFERENT LEVELS OF COFFEE PULP FERMENTED WITH *Penicillium crustosum* IN PSEUDORUMINANT DIETS.

5.5.1 ABSTRACT:

Ensiled coffee pulp was fermented in solid state with the fungus *Penicillium crustosum*. The resulting product was evaluated as a source of protein and energy in feed formulations.

New Zealand White rabbits (*Oryctolagus cuniculus*) were fed with rations isocaloric (11 060 Kj/Kg) and isoproteic (15%) containing the fermented coffee pulp in levels 5-30%. A randomized block statistical design was used with a factorial arrangement of 2 x 5. The factors were:

A. Sex: male and female.

B. Treatments: four different rations were tested. A control diet was compare with diets containing three different levels of coffee pulp products (fresh, ensiled or fermented coffee pulp).

Two groups of experiments were carried out: the first one tested a control diet against diets containing three different levels of inclusion of fermented coffee pulp (5, 10 and 15%). The second experiment tested rations containing 30% of three different coffee pulp products (fresh, ensiled and fermented pulp) against a control.

It was concluded that the inclusion of bioconverted coffee pulp at levels between 5 to 15 % did not affect the daily weight gain, voluntary feed intake and feed conversion. However rations containing 30% of FCP influenced negatively the animal performance.

6. IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER.

6.1 Impact:

The research work done at ICAITI, strengthened its capacity for development of technology for agricultural by-products utilization, mainly via bioconversion. A solid state fermenter, useful to bioconvert lignocellulosic materials, was adapted from an ribbon blender. This equipment will complement the existing facilities of the process development laboratory which is devoted to give technical assistance to the Centralamerican industry. A modern high pressure liquid chromatography equipment capable to analyze specific compounds in agroindustrial wastes was also acquired. This equipment will permit to carry out specialized analysis required in basic and applied research as well as in the service to the industry.

A strengthening of the relationship between ICAITI and the Institute of Nutrition of Central America and Panama (INCAP) was a major result. The graduated students from the San Carlos

University benefited from the support to their acquisition of research experience that led to graduation thesis

6.2 Relevance:

Utilization of agricultural wastes specifically fresh coffee pulp in animal feeding will solve partially the disposal problems created by this material and it will relief in the dry season the scarcity of pasture for ruminants . Simple treatments applied to fresh coffee pulp as screw pressing followed by silage adding additives (mainly sugar cane molasses as energy source) will improve significantly the quality the quality of the material and will reduce antiphysiological compounds. The technology developed will present different alternatives going from simple process as silage to others more elaborated as solid state fermentation. The knowledge of bioconversion of coffee pulp through solid state fermentation will open new opportunities to use such material not only as ingredient for animal rations but as support of antagonist microorganisms of soil diseases.

6.3 Technology transfer:

The findings of this research have been transferred through attendance to specialized meetings, publication of scientific papers, and technical communications oriented to the coffee grower and cattle rising sectors.

7. PROJECT ACTIVITIES/OUTPUTS.

7.1 Coordination meetings:

During the period comprised between February 18-23, 1990 the head of the project, Carlos Porres visited the Israeli counterparts in order to coordinate research activities.

On February 19-20 he visited Dr. Daniel Ben-Gedhalia at The Metabolic Unit, The Volcani Center (Bet Dagan, Israel). During the technical stay the contract between The Volcani Center and ICAITI was revised and signed.

On February 21-22, Dr. Yitzhak Hadar from the Levi Eshkol School of Agriculture, Faculty of Agriculture, The Hebrew University of Jerusalem was visited. During the visit the working plan and the contract between ICAITI and Levi Eskol School of Agriculture was discussed, revised and signed.

On October 21 to 24, 1991 Dr. Daniel Ben-Ghedalia paid a visit to ICAITI in order to present the results obtained by the two Israeli Institutions. The Volcani Center presented data regarding

digestibilities of coffee pulp and The Hebrew University the results of preliminary screening of fungi growing on coffee pulp.

7.2 Technical visits:

7.2.1 Israel:

Taking advantage of technical stay at the Israeli Institutions, the head of the project visited industries and institutions involved in themes related to the project activities:

On February 19, two industries involving animal feeding were visited:

An industry producing milk for the dairy industry was firstly visited. The point of interest in such industry was the utilization of non-traditional lignocellulosic materials to feed the milking cows. The other visit was to an industry who treated poultry droppings mixed with cassava pellets to prepare a fermented product utilized in ruminant feeding.

On February 21, industries involved in bioconversion of lignocellulosic materials for different purposes were visited. The first visit was to a mushroom factory which utilized treated wheat straw as raw material for mushroom cultivation. The second visit was to a factory producing treated substrate for the mushroom factories of the region. The last visit was to MIGAL Galilee Technological Center (kiryat Shmona) a research center dedicated between other topics to fungal research for practical applications.

7.2.2 Other countries:

On July 9, 1991 the head of the project paid a visit to Dr. Emilio Vargas from the "Centro de Investigación en Nutrición Animal", Universidad de Costa Rica, (Costa Rica). The purpose of the meeting was to evaluate the possibility of implement a joint project aimed to evaluate fermented coffee pulp with ruminants. Unfortunately the quantities required of fermented coffee pulp to prepare the rations surpassed the capacity of the ICAITI·s fermenter.

On November 4-7, 1991 the head of the project attended to the "II Seminario Internacional sobre Biotecnología en la Agroindustria Cafetera (II SIBAC)", held in Manizales Departamento de Caldas, Colombia. In such meeting a technical paper dealing with solid state fermentation of coffee pulp was presented.

On May 14, 1992 the head of the project paid a visit to the biotechnology laboratory of the Universidad Autonoma de México (UAM), (Delegación de Iztapala, Estado de México) in order to discuss advances on solid state fermentation of coffee Pulp .

7.3 Publications:

The following technical paper was public..ed in a specialized technical journal :

C. Porres, D. Alvarez and J. Calzada. 1993. Caffeine reduction in coffee pulp through silage. **Biotechnology Advances**. Vol 11, pp. 519-523.

Additional technical articles will be submitted for publication:

C. Porres, D. Alvarez and J.F. Calzada. "Reduction of antiphysiological compounds of coffee pulp through physical treatments".

C. Porres, D. Alvarez and J.F. Calzada. "Reduction of caffeine of coffee pulp through solid state fungal fermentation."

C. Porres, C. Muñoz and J.F. Calzada. "Treated and untreated coffee pulp as major component in pseudoruminant feed.

C. Porres, L.A. Calzada y J.F. Calzada. "Effect of different levels of coffee pulp fermented with *Penicillium crustosum* in pseudoruminant diets.

Three graduation thesis for university degrees were prepared:

C. E. Muñoz. 1993. "Uso de la pulpa de cafe como ingrediente en las dietas para conejos de engorde". Tesis Lic. en Zootecnia. Universidad de San Carlos de Guatemala, Facultad de Medicina Veterinaria y Zootecnia, Escuela de Zootecnia.

Calzada, L.A. 1993. "Utilización de tres niveles de biomasa residual de pulpa de café tratada con el hongo *Penicillium crustosum* en engorde de conejos". Tesis Lic. en Zootecnia. Universidad de San Carlos de Guatemala, Facultad de Medicina Veterinaria y Zootecnia, Escuela de Zootecnia.

Micheo, R. "Utilización de pulpa de café en dietas para conejos". Tesis Lic. en Zootecnia. Universidad de San Carlos de Guatemala, Facultad de Medicina Veterinaria y Zootecnia, Escuela de Zootecnia. (Unpublished thesis).

8. PROJECT PRODUCTIVITY.

All the proposed goals in the original plan were accomplished. There was a variant only in the activities regarding animal feeding tests. Original plans contemplated to evaluate the fermented product with dairy cows under the supervision of the animal science department of the University of Costa Rica. Unfortunately the required bioconverted material for such test surpassed the capacity of the ICAITI·s fermenter. For this reason the evaluation of the fermented material was carried out with rabbits under the supervision of The Institute of Nutrition of Central America and Panama (INCAP). A comparison between the proposed and executed project·s activities is presented in the next page.

8.1 Outputs of the project:

Forecasted	Actual
1. One pretreatment process developed to facilitate the reduction of the antiphysiological compounds in the coffee pulp.	Two pretreatments processes that reduce the content of antiphysiological compounds and facilitate further reduction through fermentation were developed.
2. One fungal strain capable of reducing the antiphysiological compounds selected.	The strain ***Penicillium crustosum*** reduces the caffeine content up to 90%.
3. One bioconversion process developed to reduce the antiphysiological factors in pretreated coffee pulp.	The bioconversion process developed includes fungal solid state fermentation and reduces the caffeine content 60-90%
4. A pilot plant solid state fermenter developed.	A solid state fermenter of 10 kg of capacity in operation at ICAITI.
5. Convenient levels of inclusion of bioconverted material in diet determined through animal feeding. tests.	Upper levels of inclusion of bioconverted coffee pulp as diet for pseudoruminants were determined. Adequate levels of bioconverted coffee pulp as probiotic for pseudoruminants were determined.
6. Some publications and trainings	Five technical papers were prepared. Three graduation thesis for university degrees were prepared by students working with the project.

9. FUTURE WORK.

It has been planned to continue solid state fermentations of coffee pulp with emphasis on :

1 Testing the fungus strain *Penicillium roqueforti* (utilized in production of fermented food) in order to prepare an ingredient for animal rations formulation. It is expect to minimize physiological damage observed in rabbits fed with rations containing high percentages (> 30%) of fermented coffee pulp with *Penicillium crustosum*.

2. In the related field of biological control, to prepare a suppressive material containing antagonistic microorganisms against soil diseases.

10. LITERATURE CITED

J. E. Braham.(1979). Coffee Pulp in other species. In: Coffee pulp: composition, technology and utilization. J. E. Braham and R Bressani (Eds.) IDRC publication, Ottawa, Canada, pp 39-49.

R. Bressani. (1979). Antiphysiological factors in coffee pulp. In: Coffee pulp: composition, technology and utilization. J. E. Braham and R Bressani (Eds.) IDRC publication, Ottawa, Canada, pp 83-88.

R. Jarquin (1979). Coffee pulp in swine feeding. In: Coffee pulp: composition, technology and utilization. J. E. Braham and R Bressani (Eds.) IDRC publication, Ottawa, Canada, pp 39-49.

M.T. Cabezas, A. Flores and J.J. Egaña. (1979). Use of coffee pulp in ruminant feeding, in: Coffee pulp: composition, technology and utilization, J. E. Braham and R Bressani (Eds.) IDRC publication, Ottawa, Canada, pp 71-81.

R. Gómez Brenes. (1979). "Processing of coffee pulp: chemical treatments", in: Coffee pulp: composition, technology and utilization, J. E. Braham and R. Bressani (Eds.) IDRC publication, Ottawa, Canada, pp 71-81.

11. APPENDIXES

The technical articles described in appendixes 1-5 contain the results obtained in the research project. The paper described in Appendix 1 was published in **Biotechnology Advances, volumen 11 pages 519-523, 1993.** The other articles will be submitted to specialized journals for publication.

In appendixes 6 and 7 the final reports of the Israeli counterparts are included.

11.1 Appendix 1

CAFFEINE REDUCTION IN COFFEE PULP THROUGH SILAGE (*)

11.1.1. ABSTRACT:

Silage tests to study reductions of antiphysiological compounds (caffeine and polyphenols) of fresh coffee pulp during the anaerobic fermentation were done. A concrete silo divided in compartments, with a total capacity of 9 tons of fresh material was utilized. The silage periods ranged between 98-224 days and the following materials were ensiled: 1) coffee pulp, 2) coffee pulp with sugar cane molasses, 3) coffee pulp with a mixture of molasses and ammonia and 4) screw pressed coffee pulp with molasses. Reductions in caffeine, total polyphenols and condensed polyphenols ranged between 13-63%, 28-70% and 51-81% respectively.

It was concluded that in the case of coffee pulp, silage presents and ideal method to preserve the material and partially reduce the contents of antiphysiological compounds .

11.1.2. KEYWORDS:

Coffee pulp, caffeine reduction, solid fermentation, silage, polyphenols, antiphysiological compounds.

11.1.3. INTRODUCTION:

Coffee pulp is the exocarp of ripe coffee fruit and during the coffee processing (by the wet method), it is generated in a proportion of 40% by weight of the total processed fruit. Coffee pulp is a seasonal by-product with high levels of moisture (80-82%) and fermentable sugars (total sugar 2-10% dry basis). The dried material being about 12% of crude protein, 21% of crude fiber, 8% ash, 1.25% caffeine, 1% polyphenols and 44% nitrogen-free extract.

It is estimated that 1.3 million tons of fresh pulp are produced in Central America every year during the coffee crop . A fraction of this material is utilized for compost production for coffee plants

(*)Paper published in Biotech. Adv. Vol. 11. pp 519-523, 1993.

nurseries and the rest is discharged. The disposal of the fresh material represents a serious problem for processing factories; the usual methods to discharge this material consist in dumping it into the rivers or piling it in nearby agricultural lands.

Obviously the improper disposal methods cause severe environmental pollution and the wet material with high sugar content presents an ideal substrate for wild fermentations and breeding sites for insects (mainly flies).

Several uses for this material have been proposed such as: compost production, energy generation, feed, etc. However, the relative high contents of caffeine, polyphenols and potassium have limited its utilization in animal feeding. The contents of antiphysiological compounds can be reduced significantly through biological processes (solid state fermentation, silage, etc) or physical treatments (solid-liquid extractions).

11.1.4 OBJECTIVE:

The objective of this research was to evaluate the changes in composition of fresh coffee pulp after silage.

The specific objectives were:

- To evaluate after silage the reductions in the following antiphysiological compounds: caffeine and total and condensed polyphenols.

- To evaluate after different periods of silage the variations in chemical composition of fresh coffee pulp .

- To evaluate the influence on silage of different treatments applied to coffee pulp before the process.

11.1.5 MATERIALS AND METHODS:

A rectangular concrete silo (6 m length, 1.75 m wide and 1.2 m height) with three separated compartments of 3 tons of capacity each was utilized. The fresh material was collected in a coffee plantation located 60 Km from Guatemala City at 1600 meters above sea level. Four different materials were ensiled, in different trials:

- Fresh coffee pulp.
- Fresh coffee pulp with 5% of sugar cane molasses(wet basis).
- Fresh coffee pulp with 1.25% of sugar cane molasses and 1.2% of ammonia (wet basis).
- Screw pressed fresh coffee pulp with 5% of sugar cane molasses (wet basis).

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The fresh pulp was pressed with a locally manufactured screw press with a capacity of 1 t/h. During pressing 30% of the original weight was eliminated as pulp juice. The fresh coffee pulp and the ensiled materials were analyzed for:

- Caffeine (Ishler, 1948).
- Total and condensed polyphenols (AOAC 9.110).
- Total sugars.
- In vitro digestibilities (Clarke, 1982).
- Nitrogen (AOAC 2.055).
- Lignin (Goering, 1970).
- Ash (AOAC 3.004).
- pH of silage.

In the silo, the material was manually compacted and covered with a plastic sheet and a 0.1 m thick layer of soil on the top. Woven polypropylene sacks containing samples of the original material were also placed in the silo in order to evaluate the weight losses during the process.

11.1.6 RESULTS AND DISCUSSION:

In table 1 the contents of antiphysiological compounds and ash in fresh coffee pulp and ensiled materials with different additives are shown.

Table 1

Reduction of antiphysiological compounds in coffee pulp through silage.

Sample	Caffeine (%)	Polyphenols Total (%)	Polyphenols Condensed (%)	Ash (%)
Crop 89-90				
Fresh	1.04	3.37	0.97	10.64
Ensiled (223 days)1/	0.39	1.00	0.18	8.93
Fresh	1.04	2.29	0.51	8.87
Ensiled (125 days)2/	0.68	1.64	0.25	11.57
Crop 90-91				
Fresh	0.71	1.21	0.26	9.37
Ensiled (187 days)	0.52	0.66	0.70	9.63
Ensiled (134 days)	1.31	0.91	1.03	8.78
Crop 91-92				
Fresh	0.75	1.26	0.87	7.91
Ensiled (99 days)3/	0.65	2.11	----	10.67

- Mean weight loss after silage (wet basis): 25%

1/ Fresh pulp ensiled with molasses (5%) without pressing
2/ Fresh pulp ensiled with ammonia (0.25%) and molasses without pressing.
3/ Fresh pulp screw pressed and ensiled with molasses (5%).

In general terms it could be observed that the caffeine content is reduced with silage and the extent of reduction is function of the storage time (table 1) . The reductions in caffeine ranged from 13% (silage time 99 days) to 63% (silage time 223 days).

Similar tendencies were observed with the contents of total and condensed polyphenols. The reductions were 28% (silage time: 125 days) to 70% (silage time: 223 days) for total polyphenols and 51% (silage time: 125 days) to 81% (silage time: 223 days) for condensed polyphenols.

The reductions in sugar content of ensiled materials compared with fresh pulp ranged within 50 to 93% (table 2). A moderated increase in the nitrogen content and a significant increase in the values of lignin and ash contents were observed. Lower values of pH (4.1-4.3) were obtained adding 5% of sugar cane molasses compared to those obtained with only fresh material (4.45-4.6).

Weight losses (wet basis) during silage of fresh coffee pulp without additives increased in direct proportion with the silage time. Thus for 125 days of silage the weight loss was 19.5 % and for 223 days was 26.9%.

Table 2

Variation of Chemical Composition of Coffee Pulp After Silage.

Sample	Total Sugars (%)	Nitrogen (%)	Lignin (%)	pH	Weight Loss (%)
Crop 89-90					
Fresh	3.06	2.45	15.54	ND	---
Ensiled (223 days)1/	1.38	2.51	23.71	4.6	26.9
Fresh	4.44	2.11	20.54	ND	---
Ensiled (125 days)2/	2.21	2.51	15.81	4.5	23.0
Crop 90-91					
Fresh	5.41	2.82	16.16	ND	---
Ensiled (187 days)	0.39	3.01	20.77	4.3	ND
Ensiled (134 days)	0.49	2.87	18.26	6.2	ND
Crop 91-92					
Fresh	1.97	1.85	19.14	ND	---
Ensiled (99 days)3/	0.65	2.12	-----	4.1	ND

ND: Not determined.
1/ 2 /3/ See footnotes in table 1.

11.1.7 CONCLUSIONS:

1.- The ensilage of coffee pulp significantly reduces the contents of caffeine and of total and condensed polyphenols. Within certain limits the reduction of antiphysiological compounds increases with the time of silage.

2. The addition of 5% of molasses (wet basis) and mechanical pressing of fresh coffee pulp improved the quality of the ensiled material.

3. Weight losses of fresh material during ensilage increase with silage time.

11.1.8 REFERENCES:

1. Clarke, T., Flinn, P.C. and McGowan, A.A., Grass Forage Sci., 37, 1130-1140 (1982).

2. Goering, H.K. and Van Soest, P.J., Forage Fiber Analysis, USDA Agriculture Handbook, (1970).

3. N.H. Ishler, T.P. Finucare and E. Borker, Anal.Chem., 20, 1162-1166 (1948).

11.2 Appendix 2

REDUCTION OF ANTIPHYSIOLOGICAL COMPOUNDS OF COFFEE PULP THROUGH PHYSICAL TREATMENTS:

11.2.1 ABSTRACT:

Fresh coffee pulp was submitted to different physical and physico-chemical treatments in order to reduce the content of antiphysiological compounds (caffeine, polyphenols and potassium) interfering with the utilization of this by-product as ingredient in rations for animals (mainly ruminants). The following treatments were utilized: screw pressing, steaming, aqueous extraction and extraction with a 4% ammonia solution.

With the best sequence: screw pressing/hot water extraction/screw pressing, the reductions of antiphysiological compounds from fresh coffee pulp were: caffeine 28%, total polyphenols 61%, condensed polyphenols 54%, potassium 20% and ash 25%.

The final product presented attractive qualities as feed for ruminants.

11.2.2 KEYWORDS:

Ammonia, antiphysiological, caffeine, coffee, extraction, polyphenols, pressing, pulp.

11.2.3 INTRODUCTION:

Around 1.3 millions tons of fresh coffee pulp are produced yearly in Central America during the crop season. This material is relatively rich in sugars, protein, fiber and potassium and due to the lack of adequate technologies most of this material is not utilized efficiently. A fraction of this by-product is utilized to elaborate compost for coffee plant nurseries but inadequate processes yield a final product of poor quality. On the other hand the contents of sugar and protein show that coffee pulp has a potential nutritive value as ingredient for animal rations similar to that of good quality tropical forages. However, the presence of relatively high contents of caffeine and polyphenols and high levels of lignified nitrogen limits its use.

Cabezas (1979) found that an inverse relation exists between the concentration of coffee pulp in the ration and the performance of growing and fattening cattle, which becomes more marked when the pulp concentration in the ration is greater than 20%. Jarquin (1979) determined that coffee pulp can be used at levels up to 16% in swine rations with any detrimental effect. Bressani (1973) found that even levels of 10% of coffee pulp in chick rations affected growth negatively.

The presence in coffee pulp of caffeine, tannins and other polyphenols (mainly caffeic and chlorogenic acid) and potassium have detrimental effects upon animal growing when the pulp is included in animal diets. Physical treatments, such as screw pressing or aqueous or alkaline extractions, or biological treatments, such as ensilage or solid state fermentation, may reduce the contents of undesirable compounds and improve the characteristics of coffee pulp as feed. The present research evaluates different methods to reduce some antiphysiological compounds by physical and physico-chemical treatments.

11.2.4 OBJECTIVE:

The general objective of this work was to study the effect of several physical and physico-chemical treatments on some nutritional characteristics of the fresh coffee pulp.

The specific objectives include:

- To reduce the volume and the water content of the material.
- To eliminate partially or totally the antiphysiological compounds.
- To prepare the material for a subsequent bioconversion.

11.2.5 MATERIALS AND METHODS:

11.2.5.1 Pretreatments of Fresh Coffee Pulp:

Fresh coffee pulp from a coffee processing plant located near Guatemala City (Finca San Isidro) was collected and treated at ICAITI. In the diagrams of figures 1 and 2 the different treatments which coffee pulp was submitted are described:

FIGURE 1

FLOW DIAGRAM FOR PROCESS "A".

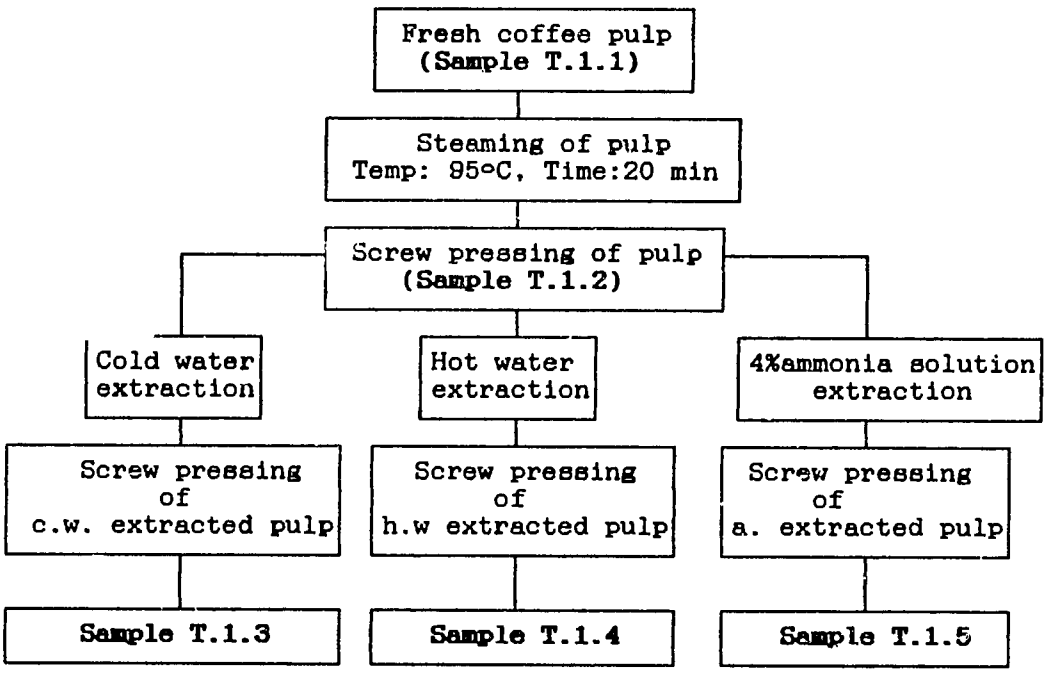
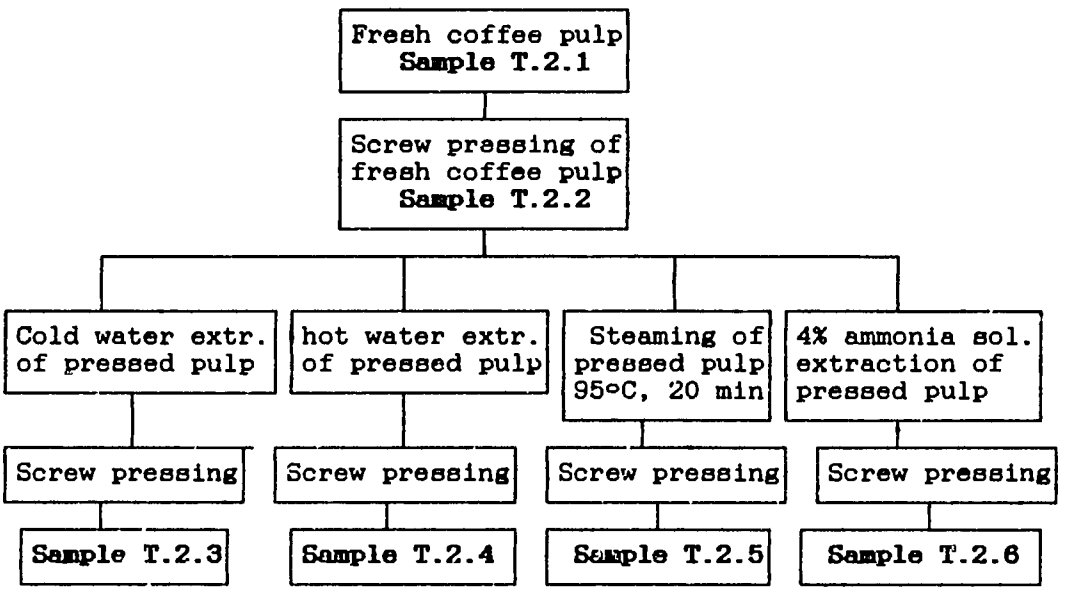


FIGURE 2

FLOW DIAGRAM FOR PROCESS "B".



The difference between the two processes was that in the case of process A an initial steaming of the material was used followed by screw pressing, and in the case of process B, only screw pressing. Subsequently treatments were quite similar for both processes.

Pressing of coffee pulp was carried out using a local manufactured screw press with a capacity of 1 ton of fresh material per hour. Steaming was carried out by placing the pulp in a metal tank (1 m^3 of capacity) with a perforated bottom and passing live steam through the material during 15 minutes. Cold and hot aqueous extractions were carried out in a rectangular tank of stainless steel (0.4 m height, 0.6 m width and 1.1 m long). Thirty liters of tap water were added to each 45.4 Kg of wet material and mixed intermittently for 30 minutes. Ammonia extractions were carried out in the same way as previously described but a 4% ammonia solution was utilized. The coffee pulp was screw pressed before and after each treatment.

Samples of original pulp and after the different treatments were taken. All the samples were analyzed for:

- Caffeine.
- Total and condensed polyphenols.
- Total sugars.
- In-vitro digestibility of dry matter and organic matter.
- Lignin.
- Nitrogen.
- Ash.
- Potassium.

11.2.6 RESULTS AND DISCUSSION:

11.2.6.1 Pretreatments of Fresh Coffee Pulp:

The contents of antiphysiological compounds of the coffee pulp samples resulting from the different treatments are shown in table 1. In table 2 the chemical composition and digestibilities of similar samples are presented.

In terms of caffeine reduction and comparing the different treatments in each process, hot water extractions yielded the highest extraction rates: 40% for process "A" and 28% for process "B". In the case of reduction of polyphenols ammonia solution extractions were the most effective treatment reducing 70% of the original content in both processes. However the extracted material still had strong ammonia odor not suitable for feed. Hot water extraction also yielded high extraction rates of polyphenols: 14% for process "A" and 61% for Process "B". The Low extraction rate of polyphenols in the aqueous stage in process "A" are due to the steaming treatment before the first pressing (see figure 1). It is suspected that high temperatures fix polyphenols to the organic material and the resulting complexes were less soluble.

Analyzing the reduction data for all antiphysiological compounds, it can be seem that the treatment screw pressing/hot water extraction/pressing (sample T.2.4 in process B) presented the most promising results. The reductions of anti-physiological compounds compared with fresh coffee pulp were: caffeine 28%, total polyphenols 61%, condensed polyphenols 54%, potassium 20% and ash 25%. The advantage of this process is that extractions were carried out at relative low solvent/solid rates: 0.66 l of liquid per kilogram of wet pressed material (3.7 l per kg of dry solids) compare to other research works which utilized ratios of solvent:solid between 10:1 to 20:1 (Gomez, 1979).

Comparing the influence of steaming the pulp before pressing, it was found that elimination of antiphysiological compounds (excepting polyphenols) and coffee pulp juice was higher in the steamed material (see tables 1 and 3). This material was easier to press at relatively high temperature (80 °C) than pulp without any treatment. It is suspected that high temperature soften lignin

and render the material more flexible and suitable to the pressing action. In terms of weight reduction after screw pressing of fresh material (expressed in wet basis), the value obtained for pulp was 22.1% and for steamed pulp 41.3%.

In relation to aqueous extraction of other components of pulp (see table 2) total sugars were extracted at higher rates in process B than process A. In both cases ammonia solutions extract less sugars than water. Nitrogen and lignin contents do not vary significantly after treatments. In the case of in-vitro organic matter and dry matter enzymatic digestibilities (IVDMED and IVOMED) of the pulps there is not a clear tendency and it can be observed that values close to the originals are maintained after treatments.

Table 1

Antiphysiological compounds in coffee pulp following physical and physico-chemical treatments.

Type of Treatment	Caffeine (%)	Polyphenols total (%)	Polyphenols condensed (%)	Potassium (%)	Ash (%)
Process "A"					
Sample T 1.1	1.10	3.60	0.71	6.02	11.8
9					
Sample T 1.2	0.90	4.10	0.58	4.88	
7.58					
Sample T.1.3	0.85	3.10	0.47	4.22	
6.52					
Sample T.1.4	0.66	3.10	0.49	4.57	
6.17					
Sample T.1.5	1.09	1.10	0.31	4.10	
6.92					
Process "B"					
Sample T.2.1	1.09	2.39	0.63	4.10	10.0
6					
Sample T.2.2	0.98	2.08	0.59	3.90	
9.51					
Sample T.2.3	0.91	1.46	0.37	3.90	
8.41					
Sample T.2.4	0.79	0.93	0.29	3.30	
7.68					
Sample T.2.5	0.85	1.86	0.42	3.60	
8.23					
Sample T.2.6	1.15	0.72	0.53	3.20	
8.62					

Process "A":

Sample T.1.1: Fresh coffee pulp without any treatment.
Sample T.1.2: Fresh pulp was steamed 10 minutes at 95 °C and screw pressed.
Sample T.1.3: The steamed and screw pressed pulp was cold water extracted and screw pressed.
Sample T.1.4: The steamed and screw pressed pulp was hot water extracted and screw pressed.
Sample T.1.5: The steamed and screw pressed pulp was extracted with a 4 % ammonia solution and screw pressed.

Process "B":

Sample T.2.1: Fresh coffee pulp without any treatment.
Sample T.2.2: Screw pressed pulp.
Sample T.2.3: Pressed pulp was extracted with cold water and pressed.
Sample T.2.4: Pressed pulp was extracted with hot water and pressed.
Sample T.2.5: Pressed pulp was steamed (10 minutes at 85 °C) and pressed.
Sample T.2.6: Pressed pulp was extracted with a 4% ammonia solution and pressed.

Table 2

Chemical composition of coffee pulp following physical and physico-chemical treatments.

Type of Treatment	Total Sugars (%)	Nitrogen (%)	IVDMKD (%)	IVOMKD (%)	Lignin (%)
Process "A"					
Sample T 1.1	7.2	2.2	28.3	30.9	20.6
Sample T 1.2	7.2	2.3	24.2	30.2	19.9
Sample T.1.3	5.6	2.3	36.1	41.8	19.6
Sample T.1.4	5.2	2.2	33.8	40.3	19.1
Sample T.1.5	3.4	4.4	30.7	39.5	18.8
Process "B"					
Sample T.2.1	14.8	2.3	35.1	36.5	23.9
Sample T.2.2	12.1	1.1	40.4	46.8	26.2
Sample T.2.3	4.2	2.3	35.9	38.3	21.9
Sample T.2.4	6.9	2.2	31.0	31.5	20.1
Sample T.2.5	5.8	1.8	31.9	44.0	25.4
Sample T.2.6	3.0	3.1	35.4	44.0	29.5

See footnotes on table 1 for key to samples.

Table 3

Moisture content and weight loss (dry basis) of coffee pulp following physical and physico-chemical treatments.

Type of Treatment	Moisture Content (%)	Weight Loss (%)
Process "A"		
Sample T 1.1	87.7	----
Sample T 1.2	81.9	13.9
Sample T.1.3	83.8	28.7
Sample T.1.4	83.6	29.7
Sample T.1.5	86.4	36.1
Process "B"		
Sample T.2.1	84.4	----
Sample T.2.2	82.0	10.3
Sample T.2.3	83.7	35.7
Sample T.2.4	81.9	36.3
Sample T.2.5	79.8	30.4
Sample T.2.6	84.1	33.7

See footnotes on table 1 for key to samples.

11.2.7 CONCLUSIONS:

1. A treatment for the fresh coffee pulp including screw pressing/hot water extraction/screw pressing presented the most promising results. The reductions of anti-physiological compounds were: caffeine 28%, total polyphenols 61%, condensed polyphenols 54%, potassium 20% and ash 25%

2. Steaming of fresh pulp improve the elimination of coffee pulp juice during pressing but it seems to fix polyphenols to organic matter, reducing aqueous extraction rate.

3. It is possible to obtain acceptable reductions in contents of caffeine and polyphenols by extracting pressed coffee pulp at relative low solid/liquid relations (0.66 l of liquid per kilogram of wet pressed material equivalent to 3.7 l per kg of dry solids).

4. Coffee pulp treated with the sequence screw pressing/hot water extraction/screw pressing produce an end product with attractive qualities for animal feeding (mainly ruminants).

11.2.8 REFERENCES:

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11.3 Appendix 3.

REDUCTION OF CAFFEINE OF COFFEE PULP THROUGH SOLID STATE FUNGAL FERMENTATION.

11.3.1 ABSTRACT:

Caffeine, polyphenols and potassium present in coffee pulp have proved their negative effects on animal metabolic performance when they are included in the diet.

The present research work was aimed to reduce caffeine and polyphenols in ensiled coffee pulp through solid state fermentation with the filamentous fungus strain *Penicillium crustosum*. The bioconverted product presented reductions of antiphysiological compounds in the following ranges, related to the fermentation time: caffeine 61-90%, polyphenols 13-61% and increments in ash 15-23%. The yield of fermented product was 89%. The reductions in dry and organic matter enzymatic digestibilities of coffee pulp were 22% and 28% respectively.

11.3.2 KEYWORDS:

Antiphysiological, caffeine, coffee, polyphenols, pulp, solid state fermentation and *Penicillium crustosum*.

11.3.3 INTRODUCTION:

The presence of caffeine, tannins and other polyphenols (mainly caffeic and chlorogenic acid) and potassium naturally occurring in coffee pulp have detrimental effects upon animal growing when it is included in the diets.

Many works have been done to eliminate these antiphysiological factors through mechanical treatments such as screw pressing followed by aqueous extractions, alkaline extractions, grinding or heating. The resulting materials present only intermediate reductions of antiphysiological compounds (Calzada and Rolz 1987, Gomez 1979).

Aquiahuatl et al (1988) cite a work done by Bergmann (1962) and Schwimmer in which a *Pseudomonas aeruginosa* strain degraded caffeine via theobromine. The same author also cite a work done by Schwimmer and Kurtzman (1972) related to the isolation and study of the metabolism of a *Penicillium crustosum* strain, which efficiently utilized caffeine via theophyline as source of nitrogen in clearly defined growth media with coffee infusions.

A laboratory research work aimed to reduce antiphysiological compounds (caffeine and polyphenols) and increase protein content

of coffee pulp through solid state fermentations with the strains: ***Aspergillus niger A-10***, ***Aspergillus oryzae*** and ***Sporotrichum pulverulentum*** is described by Porres and co-workers (1987). In relation to degradation of antiphysiological compounds in the fermented products it was concluded that the reductions of caffeine and polyphenols were: ***A. niger*** 13.2% and 3.1%, ***A. oryzae*** 23.3% and 49.7% and ***S. pulverulentum*** 54% and 46% respectively. The expected high reduction levels were not reached with these starins.

Screening tests with a similar purpose, using 26 strains of basidiomycetes growing on coffee pulp during 30 and 60 days were described by De León (1987). The strains which presented the higher rates of reductions of caffeine and polyphenols during a growing period of 60 days were: ***Phaenerochaete chrysosporium*** 53.4% and 42.3% and ***Phebia radiata*** 52.8% and 52.1 respectively. Again, the expected high reduction levels were not reached.

Aquiahuatl et al (1988) also report the results obtained from their isolation and testing of strains naturally occurring in coffee plants. According to the author 280 strains of filamentous fungi were isolated from materials coming from coffee plantations of México. From the isolated and identified strains: ***Aspergillus*** and ***Penicillium*** were predominant, but other genera could be identified as ***Fusarium***, ***Trichoderma***, ***Geotrichum*** and some ***Zygomycetes***. The paper presents a list of filamentous fungi with high capacity to degrade caffeine in liquid media containing such compound as only source of nitrogen. The best caffeine degrading strain was a ***Penicillium roqueforti*** with 95% of reduction and a degradation velocity of 0.224 mg/ml/d followed by an ***Aspergillus oryzae*** (78% and a rate of 0.157 mg/ml/d) and an ***Aspergillus sp.*** (71% and a rate of 0.12 mg/ml/d).

The present paper present the results obtained when the sustrate consisting in pre-treated (ensiled, pressed and dried) coffee pulp was fermented in solid state with the filamentous fungus strain ***Penicillium crustosum***.

11.3.4 OBJECTIVE:

The general objective of this research work was to reduce the antiphysiological compounds (caffeine and polyphenols) of coffee pulp through solid state fermentation of pretreated material with ***Penicillium crustosum***.

11.3.5 MATERIALS AND METHODS:

Fresh coffee pulp was screw pressed to reduce volume and moisture content from approximately 86% to 80%. Afterwards 5% of sugar cane molasses (wet basis) was added to the material and ensiled during

99 days. After silage the material had a pH of 4.1 and it was sun dried and stored.

The innocula were prepared in sumerged culture using 400 ml shaker flasks with 125 ml of growing medium prepared dissolving 30 g of Sabouraud dextrose broth per liter of water. The medium was sterilized at 121 °C by 15 minutes. Mycelium was obtained from cultures maintained at low temperatures (1 °C) in tubes with agar. The culture was incubated at room temperature for 3 days in a laboratory shaker. The fungi strains utilized in the work were:

Penicillium crustosum ICAITI 1159
Penicillium crysogenum ICAITI 1157
Penicillium melini ICAITI 1061

The sustrate was prepared by adding water to the sun dried ensiled coffee pulp to achieve 60% of humidity. The moisturized material was sterilized at 121 °C by 15 minutes. Eight kgs of sterile sustrate were inoculated with 375 ml of sumerged culture of the fungus. The inoculated material was fermented in a solid state fermenter.

The fermenter consisted in a stainless steel ribbon blender (0.85 m long, 0.3 m width and 0.35 m) height with a volume of approximately 9 liter and driven by a 0.77 KW motor. The fermenter had a central shaft with four paddles for mixing the material. At the bottom there was an orifice with a sliding door to discharge the product. Before each fermentation the fermenter was disinfected with solutions of chlorine and quaternary ammonium and after that the apparatus was cleaned with live steam. The solid state fermentations were carried out at semi aseptic conditions. The fermentations were maintained until sporulation was observed and this was the criteria for the fermentation time.

11.3.6 RESULTS AND DISCUSSION:

11.3.6.1 Strain selection:

The contents of caffeine and polyphenols of the following materials: sustrate and solid state fermented products with different strains are presented in table 1. These tests were aimed to select a microorganism with clear activity against caffeine and polyphenols. From the analysis of the data presented in the table it can be seen that only *Penicillium crustosum* was able to metabolize caffeine. The increase of caffeine percentage in others was due to base weight losses through metabolism. The percentages of reduction of caffeine and polyphenols after these preliminary solid state fermentations were 82% and 16% respectively. The mean fermentation time was 155 h and the yield of fermented product with respect to sustrate was 11.4%.

This screening test allowed to find a strain with high capacity to degrade caffeine.

11.3.6.2 Kinetics of caffeine and polyphenols degradation:

In table 2 the data regarding degradation of caffeine and polyphenols and concentration of ash during the fermentation time are presented.

From the analysis of data it can be seen that reductions of caffeine ranging from 77% to 86% were obtained with fermentation times between 232-311 h. Reductions in polyphenols oscillated between 38-43% and increments in ash due to concentration of inorganic material ranged 17-50%. Yields of bioconverted products varied between 88-89% with a mean value of 88.6%.

In table 3 changes in chemical composition of coffee pulp are presented.

The in-vitro dry matter enzymatic digestibility (IVDMED) and the in-vitro organic matter digestibility (IVOMED) decreased in inverse relation to fermentation time. Thus reduction in IVDMED ranged between 26 to 40% and reductions in IVOMED varied between 23% to 45% for fermentation times varying between 232-311 h. Slight increments in nitrogen and lignin due to weight losses because respiration of the fungus during fermentation were observed. At the beginning of the fermentation cycle the sugar content tend to increase up to a maximum value and then this figure was decreasing along the time.

11.3.6.3 Chemical composition of bioconverted pulp with *P. crustosum*:

Results of additional solid state fermentations carried out in order to prepare material for animal feeding tests are shown in tables 4 and 5. The results obtained are similar to those described in the previous section. The range of reductions in antiphysiological compounds were : caffeine 61-90%, polyphenols 13-61%. The ash content increased in a range of 15-23% and bioconverted product yields were approximately 89%.

The contents of total sugars, nitrogen and lignin were higher in the fermented products than in the sustrate. Digestibilities of the dry and organic matter of the fermented products were lower than those of the sustrate and the reductions were 22 and 28% respectively. In spite of reductions in digestibilities of ensiled coffee pulp after fermentation, the bioconverted product presents attractive characteristics for animal feeding: protein between 13-14%, caffeine less than 0.15%, acceptable reductions in polyphenols and in some cases slight increments in total sugar compared with the original figure.

Table 1

Selection of microorganisms with activity against antiphysiological compounds of coffee pulp

Strain	Caffeine (%)	Total Polyphenols (%)
Sustrate 1/	0.22	0.58
Fermented products:		
P. crysogenum 2/	0.32	1.12
P. melini 3/	0.43	0.77
P. crustosum 4/	0.04	0.49
Wild strain 5/	0.40	0.66

1/:Ensiled coffee pulp pressed in a screw press and extracted with cold water.
2/:Solid state fermentation with *Penicillium crysogenum* (fermentation time 216 h)
3/:Solid state fermentation with *Penicillium melini* (fermentation time 48 h).
4/:Solid state fermentation with *Penicillium crustosum* (fermentation time 155 h).
5/:Solid state fermentation with a wild strain (unidentified) (fermentation time 40 h)

Table 2

Kinetics of antiphysiological compounds degradation of coffee pulp during solid state fermentation with *Penicillium crustosum*.

Time (h)	Caffeine (%) Cont.	Red.	Total Polyphenols (%) Cont.	Red.	Ash (%) Cont.	Inc.
Experiment 1						
0.0	0.21	(0.0)	0.90	(0.0)	6.22	(0.0)
66.0	0.21	(0.0)	0.54	(40.0)	6.65	(6.9)
90.0	0.12	(42.9)	0.92	(----)	6.33	(1.8)
114.0	0.02	(90.5)	0.79	(12.2)	6.39	(2.7)
138.0	0.08	(61.9)	0.73	(18.9)	7.28	(17.4)
170.0	0.05	(76.2)	0.53	(41.1)	6.97	(12.1)
233.0	0.03	(85.7)	0.51	(43.3)	7.29	(17.2)
Experiment 2						
0.0	0.65	(0.0)	2.11	(0.0)	10.67	(0.0)
65.5	0.61	(6.2)	1.41	(33.2)	12.17	(14.1)
89.5	0.51	(21.5)	1.57	(25.6)	12.55	(17.6)
160.0	0.49	(24.6)	1.53	(27.5)	13.55	(27.0)
184.0	0.26	(60.0)	1.21	(45.7)	14.63	(37.1)
208.0	0.15	(76.9)	1.05	(50.2)	15.17	(42.2)
232.0	0.13	(80.0)	1.19	(43.6)	16.05	(50.4)
Experiment 3						
0.0	0.65	(0.0)	2.11	(0.0)	10.67	(0.0)
174.0	0.46	(29.2)	1.41	(33.2)	15.14	(41.9)
239.0	0.37	(43.1)	1.39	(34.1)	13.56	(27.1)
263.0	0.28	(56.9)	1.37	(35.1)	15.59	(46.1)
287.0	0.16	(75.4)	1.27	(39.8)	13.51	(26.6)
311.0	0.15	(76.9)	1.31	(37.9)	13.26	(24.3)

Abbreviations:
Cont.: content
Red.: Percentage of reduction with respect to initial value.
Inc.: Percentage of increment with respect to initial value

Table 3

Changes in chemical composition of coffee pulp during solid state fermentations with *Penicillium crustosum*.

Type of Treatment	Total Sugars (%)	Nitrogen (%)	IVDMKD (%)	IVOMKD (%)	Lignin (%)
Experiment 1					
0.0	0.83	2.05	27.18	30.98	26.22
66.0	2.83	1.91	24.81	31.09	26.78
90.0	1.68	2.04	22.34	22.41	23.51
114.0	1.46	2.21	17.33	18.05	31.06
138.0	1.16	2.27	20.52	21.31	28.55
170.0	1.08	2.32	19.81	22.11	31.54
233.0	0.91	2.43	20.02	21.01	31.33
Experiment 2					
0.0	1.51	2.12	21.37	28.66	24.41
65.5	ND	ND	18.88	22.63	ND
89.5	ND	2.31	27.35	34.5	ND
160.0	1.97	3.08	23.54	27.88	23.73
184.0	ND	2.16	21.76	23.22	ND
208	1.17	2.84	21.66	22.10	24.21
232.0	0.71	3.15	18.82	28.87	22.91
Experiment 3					
0.0	1.51	2.12	21.37	28.66	ND
174.0	1.88	1.41	16.95	23.13	23.49
239.0	2.65	2.21	18.42	18.95	22.49
263.0	1.96	2.42	10.11	14.43	24.08
287.0	1.52	2.45	9.81	13.75	30.86
311.0	1.31	2.53	12.85	15.86	21.59

Table 4
Contents of antiphysiological compounds in bioconverted coffee pulp with *Penicillium crustosum* in 3 pilot assays.

Fermentation Time (h)	Caffeine (%)	Total Polyphenols (%)	Ash (%)	Yield (%)
Sustrate:				
0.0	0.31	0.90	6.04	---
Bioconverted products:				
137.0	0.12	0.78	7.40	89.0
142.0	0.04	0.35	7.13	89.0
168.5	0.03	0.62	6.97	88.1

Table 5
Chemical composition of bioconverted coffee pulp with *Penicillium crustosum* in 3 pilot assays.

Fermentation time (h)	Total Sugars (%)	Nitrogen (%)	IVDMKD (%)	IVOMKD (%)	Lignin (%)
Sustrate:					
0.0	0.49	2.22	27.18	30.98	25.65
Bioconverted products:					
137.0	1.02	2.43	20.22	20.49	nd
142.0	1.01	2.13	22.91	23.89	29.63
168.5	1.41	2.53	20.91	22.28	27.58

11.3.7 CONCLUSIONS:

1. The reductions of anti-physiological compounds through solid state fermentation of mechanical treated coffee pulp with *Penicillium crustosum* vary in the following ranges: caffeine 77-86% and total polyphenols 38-43%. Increments in ash in the bioconverted product vary between 17-50%.

2. The yield of bioconverted product from ensiled coffee pulp was 89%. Contents of lignin, total sugars and nitrogen in bioconverted product tend to increase compared to sustrate.

3. *Penicillium crustosum* on ensiled coffee pulp inoculated from submerged standard media cultures sporulated within a range of 130-170 hours.

4. Coffee pulp bioconverted with the fungus *Penicillium crustosum* produce an end product with promising characteristic for animal feeding (mainly ruminants).

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11.4 Appendix 4.

TREATED AND UNTREATED COFFEE PULP AS MAJOR COMPONENT IN PSEUDORUMINANT FEED.

11.4.1 ABSTRACT:

Animal feeding test with white New Zealand rabbit aimed to test two levels of inclusion of coffee pulp (15% and 40%) in the diet were done.
The following types of substitution products were tested: a) fresh coffee pulp, b) ensiled and pressed coffee pulp, c) ensiled and water extracted coffee pulp and e) fungal fermented coffee pulp with *Penicillium crustosum*.

It was concluded that the inclusion of 15 % of the above mentioned materials in different diets did not result in any significant difference ($P<0.05$) with respect to the control group.

11.4.2 KEYWORDS:

Antiphysiological, feed, caffeine, coffee, *Penicillium crustosum*, polyphenols, pseudoruminant, pulp, rabbit and solid state fermentation.

11.4.3 INTRODUCTION:

The presence of caffeine, tannins and other polyphenols (mainly caffeic and chlorogenic acid) and potassium naturally occurring in coffee pulp have detrimental effects upon animal growing when it is included in the diets.

Caffeine might be responsible for the decrease in nitrogen retention, in view of its recognized diuretic effect and the evidence that increments in urine excretion increase nitrogen losses via the same route. Moreover, caffeine presents a lypolitic action in animals and accelerates the basal metabolic rates, which might be responsible of the nervousness in animals fed coffee pulp. The increases in potassium might affect the ionic balance in the tissues. The low digestibility of pulp protein can be attributed to the formation of polyphenol complexes that incorporate themselves into the fraction analyzed as lignin (Cabezas, 1979).

Many works have been done to eliminate these antiphysiological factors via mechanical or biological methods (Calzada and Rolz 1987, Gomez 1979 and Porres et al, 1993). The resulting materials present attractive reductions of antiphysiological compounds, but the effect on growth behaviour should be studied.

Rabbit feeding tests with two substitution levels (15% and 40%) using four different coffee pulp products in the diet are described.

11.4.4 OBJECTIVE:

The general objective of this work was to study the response of rabbits (as pseudoruminants) to diets containing four different coffee pulp products, at two levels of substitution: 15% and 40%.

11.4.5 MATERIALS AND METHODS:

Materials:

Five different diets were formulated, as follow:

- Treatment 1: diet without coffee pulp (control).
- Treatment 2: diet containing 15% of dry fresh pulp.
- Treatment 3: diet containing 15 % of ensiled, pressed and dried coffee pulp.
- Treatment 4: diet containing 15% of ensiled, aqueous extracted, pressed and dried coffee pulp.
- Treatment 5: diet containing 15% of ensiled and bioconverted coffee pulp.

The diets offered to the animals were isocaloric (10 420 Kj/Kg) and isoproteic (17%). All the diets were pelletized. One feeder and one automatic waterer per cage were used. Animals were fed once a day and after 24 hours the surplus feed was weighted.

Experimental design:

A randomized block statistical design was used with a factorial arrangement of 2 x 5. The factors were:

A. Sex:
- A.1 male.
- A.2 female.

B Treatment:
- B.1 control.
- B.2 fresh coffee pulp.
...- B.3 ensiled and pressed coffee pulp.
- B.4 ensiled and aqueous extracted coffee pulp.
...- B.5 fungal fermented coffee pulp (with *Penicillium crustosum*).

It was made two blocks of samples because the factors light and wind caused difference between blocks. The experimental unit

consisted in one cage and the subsample one white New Zealand rabbit (five rabbits per cage) with an initial weight averaging 1 000 g and 45 days of age.

Development of the experiment:

One hundred white New Zealand rabbits coming from different litters were utilized and confined in twenty cages. The adaptation period was eight days. The feed consumption was recorded daily and the animals were weighted weekly. The animal feeding experiment lasted 45 days. After that, the data collected were submitted to a statistical analysis.

The statistical model utilized was the following:

$$Y_{ijkl} = \mu + \Gamma_i + S_j + (\Gamma S)_{ij} + B_k + C(X_{ij}-X_{..}) + \epsilon_{ijk} + \Omega_{ijkl}$$

Where:

Y_{ijkl}= the result (response) of the trial
μ= the grand mean of all responses
Γ_i=effect of treatment i
S_j= effect of sex
$(\Gamma S)_{ij}$=effect of interaction between treatment and sex
B_k= effect of block
$C(X_{ij}-X_{..})$= effect of co-variable
ϵ_{ijk}= experimental error in the i, j, kth trial
Ω_{ijkl}=effect of the i, j, k, lth sample
i= 1,2,3,4,5 treatments
j= 1,2 sex
k= 1,2 blocks
l= 1,2,3,4,5 samples

The variables analyzed with this model were final weight (g) and weight gain (g).

Since the feed consumption only was recorded for experimental units (cages), this variable along with the variable feed conversion were analyzed with a covariance analysis for a randomized block design.

The covariable utilized was initial weight because when it was submitted to a analysis of variance it was found that differed between treatments

11.4.6 RESULTS AND DISCUSSION:

In table 1 and 2 the contents of antiphysiological compounds and the chemical composition of the different types of coffee pulp utilized in the feeding tests are shown.

In table 3 the growth response of New Zealand rabbits to different treatments are shown.

Regarding the final weight of animals the statistical analysis showed that exist significant differences ($P<0.05$) between sex but not among the five different treatments. These results are in accordance with those presented by Alfaro et. al.(1987) who reported significant differences between sexes, females presenting a better response.

The highest final weight (2 504.4 g) was obtained with the diet containing ensiled and aqueous extracted coffee pulp. Regarding to the diet containing the bioconverted coffee pulp, it presented a final weight consumption (2 403.6 g) quite similar to the control diet (2 441.4 g).

In respect to weight gain, significant differences exist between sex ($P<0.05$) but not among treatments. The average weight gain in males were 29.5 g/d and in females 31.2 g/d presenting the last group a better performance. Although statistical analysis did not show significant differences among treatments, there was a tendency for better performance of the animals fed ensiled and aqueous extracted coffee pulp .

It was found significant differences ($P<0.05$) in feed consumption between sexes, and females presented better performance. No significant differences were observed among treatments but again there is a tendency indicating that ensiled and aqueous extracted coffee pulp presented the best response.

From the statistical analysis it was found that feed conversion does not present significant differences ($P<0.05$) neither between sex nor among treatments. The feed conversions obtained ranged between 4.79:1 to 4.89:1 .

Table 1

Contents of antiphysiological compounds in different samples of coffee pulp utilized in animal feeding tests.

Sample	Caffeine (%)	Total Polyphenols (%)	Potassium (%)	Ash (%)
Sun dried fresh pulp	0.51	0.89	4.40	9.85
Ensiled pulp	0.70	1.76	4.85	10.23
Ensiled/ aqueous extracted pulp	0.31	0.53	1.80	6.04
Bioconverted pulp	0.36	1.55	5.45	13.28

Table 2

Chemical composition of different samples of coffee pulp utilized in animal feeding tests.

Sample	Total Sugars (%)	Nitrogen (%)	IVDMKD (%)	IVOMKD (%)	Lignin (%)
Sun dried fresh pulp	0.81	1.48			21.53
Ensiled pulp	0.49	2.58			21.18
Ensiled/aqueous extracted pulp	0.48	1.80			25.07
Bioconverted pulp	0.46	2.74			29.91

Table 3

Growth response in New Zealand rabbits fed different levels of coffee pulp products for 45 days.

		Treatments					
		1	2	3	4	5	Mean
Final Weight (g)	male	2469	2290	2471	2399	2378	2401
	Female	2414	2416	2504	2610	2429	2475
	mean	2441ns	2353ns	2488ns	2504ns	2404ns	
Weight Gain (g)	male	1393	1223	1397	1319	1309	1328
	female	1338	1342	1432	1538	1360	1401
	mean	1366ns	1283ns	1414ns	1428ns	1334ns	
Feed Consupt. (g/d)	male	144	141	141	154	142	144
	female	149	146	151	156	145	149
	mean	147ns	143ns	146ns	155ns		143ns
Feed Convers. (g/g)	male	4.7	5.2	4.5	5.3	4.9	4.9
	female	5.0	4.9	4.7	4.6	4.8	4.8
	mean	4.8ns	5.0ns	4.6ns	4.9ns		4.8ns

Abbreviations:
ns: not significant.

Treatment 1: diet without coffee pulp.
Treatment 2: diet containing 15% of sun dried fresh coffee pulp.
Treatment 3: diet containing 15% of coffee pulp submitted to the following treatments: ensilage, screw pressing and sun drying.
Treatment 4: diet containing 15% of coffee pulp submitted to the following treatments: ensilage, screw pressing, aqueous extraction and sun drying.
Treatment 5: diet containing 15% of coffee pulp submitted to the following treatments: ensilage, screw pressing, solid state fermentation and drying.

11.4.7 CONCLUSIONS:

1 White New Zealand rabbits fed with four different diets, each containing 15% of one of the following materials: fresh coffee pulp, ensiled and pressed coffee pulp, ensiled and aqueous extracted coffee pulp and fungal fermented coffee pulp with *Penicillium crustosum*, did not result in any significant difference ($P<0.05$) in weight gain with respect to control group.

2 Statistical analysis show significant differences ($P<0.05$) in relation to final weight, weight gain and feed consumption between sexes. Females animals presented the best performance.

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11.5 Appendix 5.

EFFECT OF DIFFERENT LEVELS OF COFFEE PULP FERMENTED WITH *Penicillium crustosum* IN PSEUDORUMINANT DIETS.

11.5.1 ABSTRACT:

Ensiled coffee pulp was fermented in solid state with the fungus *Penicillium crustosum*. The resulting product was evaluated as a source of protein and energy in feed formulations.

New Zealand White rabbits (*Oryctolagus cuniculus*) were fed with rations isocaloric (11 060 Kj/Kg) and isoproteic (15%) containing the fermented coffee pulp in levels 5-30%. A randomized block statistical design was used with a factorial arrangement of 2 x 5. The factors were:

A. Sex: male and female.

B. Treatments: four different rations were tested. A control diet was compare with diets containing three different levels of coffee pulp products (fresh, ensiled or fermented coffee pulp).

Two groups of experiments were carried out: the first one tested a control diet against diets containing three different levels of inclusion of fermented coffee pulp (5, 10 and 15%). The second experiment tested rations containing 30% of three different coffee pulp products (fresh, ensiled and fermented pulp) against a control.

It was concluded that the inclusion of bioconverted coffee pulp at levels between 5 to 15 % did not affect the daily weight gain, voluntary feed intake and feed conversion. However rations containing 30% of FCP influenced negatively the animal performance.

11.5.2 KEYWORDS:

Antiphysiological, feed, caffeine, coffee, *Penicillium crustosum*, polyphenols, pseudoruminant, pulp, rabbit and solid state fermentation.

11.5.3 INTRODUCTION:

Coffee pulp is the exocarp of ripe coffee berries. Its disposal during the processing of the fruit (by the wet method) represents a serious environmental problem. The presence of relatively high contents of antiphysiological compounds (caffeine, polyphenols, and potassium) in fresh coffee pulp have limited its utilization in animal nutrition (mainly ruminants).

Although the Centralamerican region is a humid area, during the dry season some places suffer scarcity of forage. On the other hand, coffee pulp presents attractive contents of protein (12% in dry basis) and total sugars (up to 10% in dry basis) that make this material useful for feeding ruminants.
The main advantage of industrialization of fresh coffee pulp is to reduce the problem created by the non-technical methods of disposal of this by-product. Currently this material is dumped in water bodies or ravines.

The present paper describes the results obtained in rabbit feeding tests with different rations containing 5 to 30% of fermented coffee pulp (with *Penicillium crustosum*) compared with a control diet.

11.5.4 OBJECTIVE:

The objective of this work was to evaluate the effect of inclusion at different levels of ensiled and fermented coffee pulp (with *Penicillium crustosum*) in the diet of rabbits (as pseudoruminant models).

11.5.5 MATERIALS AND METHODS:

a) Experiments with diets containing low levels (5-15%) of fermented coffee pulp:

Materials:

Fermented coffee pulp (FCP) was prepared as follows: fresh coffee pulp was screw pressed (in order to reduce its volume and moisture content), enriched with 5% of sugar cane molasses and ensiled in a concrete silo. After three months, the silo was emptied and the ensiled material was sun dried (on a concrete surface) and stored.

The dry ensiled coffee pulp was then moisturized to 60 % of water content, sterilized at 121 °C for 15 minutes and fermented in solid state with the fungus *Penicillium crustosum*. The bioconverted material was dried (to 15% of humidity) at a temperature of 30 °C, on a tray dryer .

Different diets were formulated, as follow:

- Treatment 1: control.
- Treatment 2: diet containing 5% of FCP.
- Treatment 3: diet containing 10% of FCP.
- Treatment 4: diet containing 15% of FCP.

The diets offered to the animals were isocaloric (11 060 Kj/Kg) and isoproteic (15%). All the diets were pelletized. Sixteen cages of 2 m long, 0.6 m wide and 1 m height with concrete floor were

utilized. Each cage was equipped with one feeder, one automatic waterer and one lamp with a 60 watts bulb.

Methods:

Experimental design:

A randomized block statistical design was used with a factorial arrangement of 2 x 4. The factors were:

A. Sex:
- A.1 male.
- A.2 female.

B Treatment:
- B.1: control.
- B.2: diet containing 5% of FCP.
- B.3: diet containing 10% of FCP.
- B.4: diet containing 15% of FCP.

Two blocks of samples it was made because the factors light and wind caused differences between blocks. The experimental unit consisted in one cage (five rabbits per cage) and the different groups were balanced with respect to initial weight and sex.

Development of the experiment:

Eighty white New Zealand rabbits (forty males and forty females) coming from homogenous litters were utilized and confined in sixteen cages (five animal per cage). The adaptation period was eight days. The feed consumption was recorded every three days and the surplus feed was weighted. Animals were fed every three days and the animals were weighted weekly.

The animal feeding experiment lasted 52 days and after that, the data collected were submitted to a statistical analysis. The statistical model utilized was the following:

$$Y_{ijk} = M + A_i + B_j + (AB)_{ij} + R_k + E_{ijk}$$

Where:

Y_{ijk} = the result (response) of the trial.
M = the grand mean of all responses.
A_i= effect of sex.
B_j= effect of treatments.
$(AB)_{ij}$= effect of interaction between factors.
R_k= effect of blocks or replications.
$C(X_{ij}-X..)$= effect of co-variable.
E_{ijk}= experimental error in the i, j, kth trial.

i= 1,2 (sex).
j= 1,2,3 and 4 (treatments).
k= 1,2 (samples).

The variables analyzed with this model were initial and final weight (g), weight gain (g), voluntary feed intake (g) and feed conversion.

b) Experiments with 30% of fermented coffee pulp:

In order to study the effect of inclusion in rabbit diets of high levels of fermented coffee pulp and to compare it with other similar materials (fresh pulp and ensiled pulp) the following diets were formulated:

- Treatment A: control
- Treatment B: diet containing 30% of ensiled coffee pulp.
- Treatment C: diet containing 30% of fresh coffee pulp.
- Treatment D: diet containing 30% of **FCP**.

The diets offered to the animals were isocaloric and isoproteic.

The methodology of the animal feeding tests was the same to that described in the previous section.

A randomized block statistical design was used with a factorial arrangement of 2 x 4. The factors were:

A. Sex:
- A.1 male.
- A.2 female.

B Treatment:
- B.1: control.
- B.2: diet containing 30% of ensiled pulp.
- B.3: diet containing 30% of fresh pulp.
- B.4: diet containing 30% of fermented pulp (**FCP**).

The statistical analysis and the mathematical model utilized were similar to that described previously.

11.5.6 RESULTS AND DISCUSSION:

Tables 1 and 2 present the contents of antiphysiological compounds and the chemical composition of three coffee pulp materials:

- Fresh coffee pulp.
- Ensiled coffee pulp (substrate utilized to prepare solid state fermented pulp).
- Solid state fermented pulp.

In table 3 the growth response of New Zealand rabbits to rations containing 5% to 15% of fermented coffee pulp is shown.

In table 4 the performance of rabbits fed three different rations containing 30% of ensiled coffee pulp, 30% of fresh coffee pulp and 30% of fermented coffee pulp (FCP) respectively, is presented.

11.5.6.1 Chemical composition of coffee pulp products:

In relation to the contents of antyphysiological compound in coffee pulp products, it was observed that after solid state fermentation of ensiled coffee pulp with *Penicillium crustosum*, the caffeine content was reduced in 85% . The other antiphysiological compounds did not vary significantly (see table 1).

Regarding to variation in chemical composition of ensiled coffee pulp after fermentation it can be concluded that total sugar decreased 52%, lignin content increased (12%) due to weight loss of the sustrate and nitrogen content present a slight increment (see table 2).

11.5.6.2 Animal feeding tests with rations containing 5 to 15% of fermented coffee pulp:

a) Initial and final weight:

The statistical analysis of the initial weight of animals showed that significant differences ($P<0.05$) did not exist between sex, among treatments or blocks (see table 3). Males presented higher initial weights (665 g) than females (624) and this tendency was also observed in the final weights.

Although statistical analysis did not show significant differences in relation to final weights among the different treatments, there was a tendency for higher final weights of the animals fed with rations containing fermented coffee pulp. The highest weight gain were presented by the group of animals feed with rations containing 5% of fermented coffee pulp (2 622 g). In general terms, males presented higher final weights (2 575 g) than females (2 477 g).

For comparison it can be mentioned that several authors have established that after a period of twelve weeks the final weight of

rabbits fed with balanced rations will range between 2 to 2.5 kg (Shimada (1983) and Templeton (1976)).

b) Daily and total weight gains:

In respect to daily and total weight gains, significant differences did not exist between sex (P<0.05) or among treatments. The average daily and total weight gains in males were 32.7 g/d and 1 702 g and in females 33.5 g/d and 1743 g respectively, presenting the last group a better performance.

Although statistical analysis did not show significant differences among treatments, again there was a tendency for better performance of the animals fed rations containing 5 % of ensiled and fermented coffee pulp.

c) Total feed consumption:

Significant differences (P<0.05) were not found in feed consumption between sexes; females presented a consumption slightly higher compared to males (6 737 g against 6 686 g). It was observed a tendency indicating a greater feed consumption as the content of fermented coffee pulp in the ration increased.

e) Feed conversion:

From the statistical analysis it was found that feed conversion does not present significant differences (P<0.05) between sex or among treatments but there was a decreasing tendency showing a lower feed conversion as the presence of fermented coffee pulp in the ration increased. In general terms it can be affirmed that 3.7 kg of the control ration were necessary to increase one kg of animal weight compared to 4.1 kg of the ration containing 15% of fermented coffee pulp.

11.5.6.3 Animal feeding tests with rations containing 30% of fermented coffee pulp:

From the statistical analysis significant differences (P>0.05) were not found among the different treatments in reference to weight gain, total feed consuption and feed conversion (see table 4). However, it was observed a reduction tendency in animal performance in the treatment including 30% of fermented coffee pulp in the diet.

Some internal lesions in trachea and lungs were observed in animals fed with rations containing 30 % of **FCP**, but none in the case of 5%-15% of **FCP**.

Table 1

Contents of antiphysiological compounds in different samples of coffee pulp utilized in animal feeding tests. (Dry basis).

Sample (%)	Caffeine (%)	Polyphenols Total (%)	Polyphenols Condensed (%)	Potassium (%)	Ash
Fresh pulp	0.65	1.04	0.10	4.40	11.60
Ensiled pulp	0.78	1.74	0.27	4.85	17.06
Bioconverted pulp	0.12	1.80	0.41	5.45	14.23

Table 2

Chemical composition of different samples of coffee pulp utilized in animal feeding tests. (Dry basis).

Sample	Total Sugars (%)	Nitrogen (%)	IVDMKD (%)	IVOMKD (%)	Lignin (%)
Fresh pulp	1.36	2.46	24.07	30.44	31.66
Ensiled pulp	2.60	3.90	28.08	33.39	31.44
Bioconverted pulp	1.26	3.36			35.13

IVDMKD: In-vitro dry matter enzimatic digestibility.
IVOMKD: In-vitro organic matter enzimatic digestibility.

Table 3

Growth response in New Zealand rabbits fed diets containing 5 to 15% of fermented coffee pulp for 45 days.

		Treatments				
		1	2	3	4	Mean
Initial Weight (g)	male	606	675	689	696	666(ns)
	female	627	629	602	637	624(ns)
	mean	617	652	645	666	
Final Weight (g)	male	2458	2783	2571	2488	2575(ns)
	female	26343	2461	2542	2590	2559(ns)
	mean	2550(ns)	2622(ns)	2556(ns)	2539(ns)	
Weight Gain (g)	male	1665	1855	1687	1599	1702(ns)
	female	1805	1629	1775	1768	1744(ns)
	mean	1735(ns)	1742(ns)	1731(ns)	1684(ns)	
Daily Weight Gain (g)	male	32.0	35.7	32.5	30.8	32.7(ns)
	female	34.7	31.3	34.1	34.0	33.5(ns)
	mean	33.4(ns)	33.5(ns)	33.3(ns)	32.4(ns)	
Tot.Feed Consupt. (g/d)	male	6139	6710	6829	7067	6686(ns)
	female	6835	6480	6868	6763	6737(ns)
	mean	6487(ns)	6595(ns)	6849(ns)	6915(ns)	
Feed Convers. (g/g)	male	3.7	3.6	4.1	4.5	4.0(ns)
	female	3.8	4.0	3.9	3.8	3.9(ns)
	mean	3.8(ns)	3.8(ns)	4.0(ns)	4.2(ns)	

Abbreviations:
ns: not significant (P>0.05).

Treatment 1: control.
Treatment 2: diet containing 5% of **FCP**.
Treatment 3: diet containing 10% of **FCP**.
Treatment 4: diet containing 15% of **FCP**.

Table 4

Growth response in New Zealand rabbits fed rations containing 30% of coffee pulp products for 45 days.

		Treatments			
		1	2	3	4
Initial Weight (g)	male	1 193	1 250	1 191	1 325
	female	1 243	1 243	1 250	1 134
	mean	1 218 ns	1 246 ns	1 220 ns	1 230 ns
Final Weight (g)	male	2 814	2 803	2 794	1 591
	female	2 708	2 782	2 681	2 519
	mean	2 761 ns	2 792 ns	2 738 ns	2 555 ns
Weight Gain (g)	male	1 621	1 553	1 603	1 266
	female	1 425	1 539	1 431	1 385
	mean	1 543 ns	1 546 ns	1 517 ns	1 326 ns
Daily Consupt. (g/d)	male	101	119	114	100
	female	110	114	105	97
	mean	106 ns	116 ns	110 ns	98 ns
Feed Convers. (g/g)	male	3 738	4 598	4 267	4 739
	female	4 505	4 288	4 402	4 202
	mean	4 122 ns	4 443 ns	4 334 ns	4 470 ns

Abbreviations:
ns: not significant (P>0.05).

Treatment 1: control.
Treatment 2: diet containing 30% of ensiled coffee pulp.
Treatment 3: diet containing 30% of fresh coffee pulp.
Treatment 4: diet containing 30% of FCP.

11.5.7 CONCLUSIONS:

1 Utilization of ensiled and fermented (with ***Penicillium crustosum***) coffee pulp in levels up to 15% in rations for White New Zealand rabbits did not result in any significant difference ($P<0.05$) in daily and total gain weight, feed consumption and feed conversion with respect to control group. However rations containing 30% of FCP influenced negatively the animal performance.

2 Although statistical analysis did not show significant differences in relation to daily and total gain weights among the different treatments, there was a tendency for a better response of the animals fed with rations containing 5% of fermented coffee pulp.

3. Ingredients commonly used in animal nutrition (like maize or soy bean flour) can be substituted with fermented coffee pulp in levels up to 15 % and then those can be released for more attractive uses (such as human consumption).

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11.6 Appendix 6.

"Final report of The Hebrew University of Jerusalem (Israel)".

SCIENTIFIC REPORT

submitted to

ICAITI-GUATEMALA

by

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INTRODUCTION

Coffee pulp is an important agro-industrial byproduct in Guatemala and generally in coffee producing countries. Animal feeding appears to be the most economic target-use. However, some intrinsic factors hamper its use as an animal fodder; coffee pulp is high in lignin, phenolics and caffeine. Rumen microflora lacks the conditions and the enzymatic (oxidation) machinery required to cope with lignin, which is regarded as a major biodegradation obstacle. Phenolics and caffeine are known to exert detrimental effects both on rumen microflora and on the host animal. Therefore, a biologicai treatment of the pulp prior to feeding, aimed at reducing thelevel of anti-nutritional factors, would be beneficial. Various strains of fungi were examined and screened and the most successful ones were chosen for further analyses.

Solid state fermentation of coffee pulp was studied along with studies on effect of aromatic compound on fungal growth and activity.

A. Fungal Growth on Coffee Pulp

1. List of fungi that were used:

Pleurotus ostreatus (F6)
Phanerochaete chrysosporium (BKM)
Schizophyllum commune
Gleophyllum striatum
Coriolus versicolor (Trametes)
Coriolopsis polyzona
Phlebia tremellosus
Polyporus anceps
Poria medulla
Ganoderma applantum

2. Spawn preparation:

900 ml distilled water were added to 1 kg of Sorghum seeds. This was autoclaved in an open Erlenmeyer flask for 1 hour. After it reached room temperature, 100 gr of $CaSO_4$ and 10 gr of $CaC0_3$ were added and mixed well. From these mixtures, 50 gr of Sorghum seeds were put into 100 ml Erlenmeyer flasks and after 24 hours they were autoclaved again for 1 hour. Each Erlenmeyer flask was inoculated with 10 agar disks containing hyphae of fungi. The Erlenmeyer falsks were then incubated in a suitable temperature for each fungi for the amount of time that was needed.

3. Coffee pulp preparation

Dry coffee pulp was mixed with distilled water to give about 50% dry matter. The coffee pulp was autoclaved for 1 hour as one batch and then divided into small amounts and put into bags or bottles. After 24 hours they were autoclaved again for 1 hour. Screening was performed on 10 gr pulp dry weight. For digestibility experiment and chemical analysis batches of 50-100 gr dry weight were prepared.

4. Coffee pulp inoculation

The coffee pulp was aspetically inoculated with approximately 5 gr of spawn (depending on coffee pulp amount). The inoculated coffee pulp was incubated in the suitable temeprature for each fungi. When fungi growth was massive, the coffee pulp was shaken so that fungi hyphae will spread all over the material. Dry weight measurement were made at the beginning and at the end of the growth period, usually a period of 3-4 weeks.

5. Most of the coffee pulp received from Guatemala was used for preparation of samples for animal nutrition experiments and were shipped to Dr. D. Ben-Gedalia at the Volcani Institute (see separate report).

6. Screening for fungal growth on coffee pulp

The fungi mentioned above were tested on a small scale. Table 1 shows the ability to grow on coffee pulp.

Table 1: The ability of different fungi to grow on coffee pulp

Fungi strains	**Growth rate**	**Growth temperature**
Pleurotus ostreatus (F6)	+++	30°C
Phanerchoaete chrysosporium (BKM)	++	38°C
Schizophyllum commune	+++	27°C
Gleophyllum striatum	++	27°C
Coriolus versicolor (Trametes)	+	30°C
Coriolopsis polyzona	+	27°C
Phlebia tremellosus	-	30°C
Polyporus anceps	-	30°C
Poria medulla	-	30°C
Ganoderma applantum	-	30°C

* +++ - very quickly, ++ - quickly, + - slowly, - no growth

8. According to what we found in the screening test we chose to grow the fastes growing fungi on a larger scale, i.e., *Pleurotus ostreatus* (F6). *Phanerochaete chrysosporium* (BKM), *Schizophyllum commune, Gleophyllum striatum.* Table 2 shows data on dry weight loss at the end of the growth period.

Table 2: Dry weight loss (average of 3-5 replicates)

Fungi strains	**Percentage**
Pleurotus ostreatus (F6)	21.31
Phanerochaete chrysosporium (BKM)	-
Schizophyllum commune	28.96
Gleophyllum striatum	28.97

The fermentation product was further analysed by Dr. Ben-Ghedalia

8. Scan Electron Microsocpy

Samples of untreated and treated coffee pulp were taken for SEM observation and are presented in the following photographs:

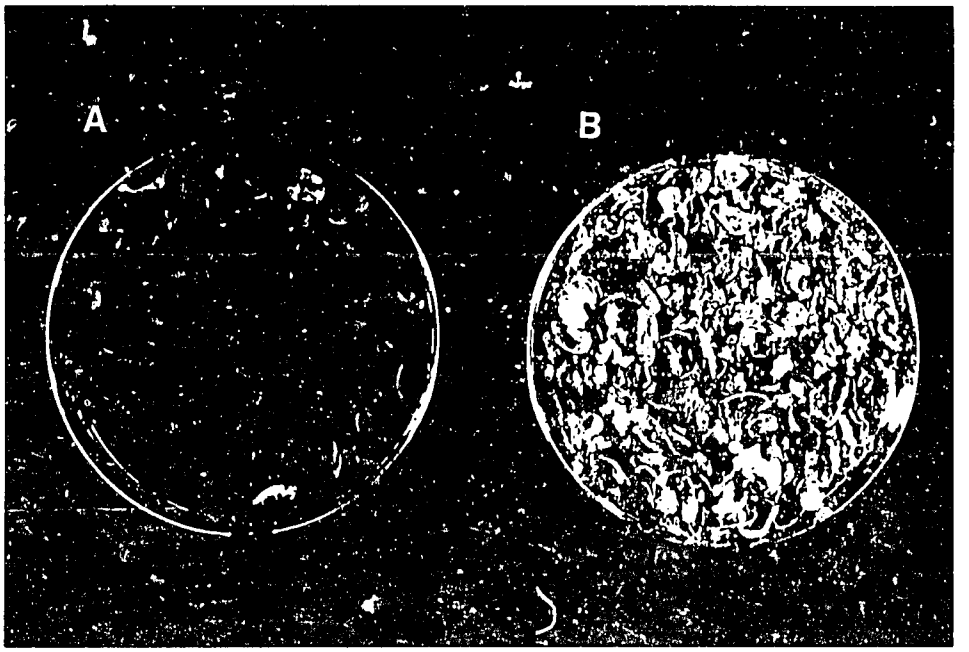

Fig. 1: Uninoculated coffee pulp (A), and coffee pulp inoculated with *Schizophyllum commune* (B)

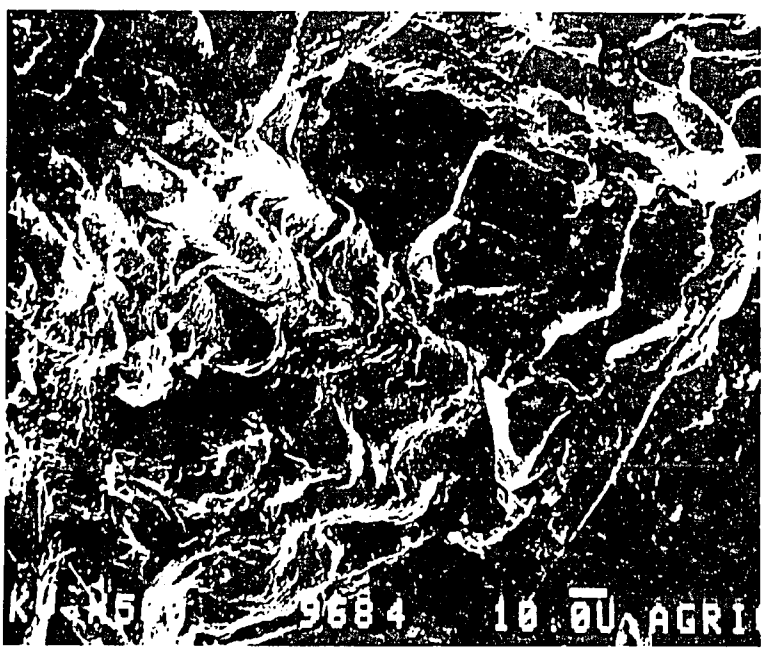

Fig. 2: Fresh coffee pulp (not inoculated)

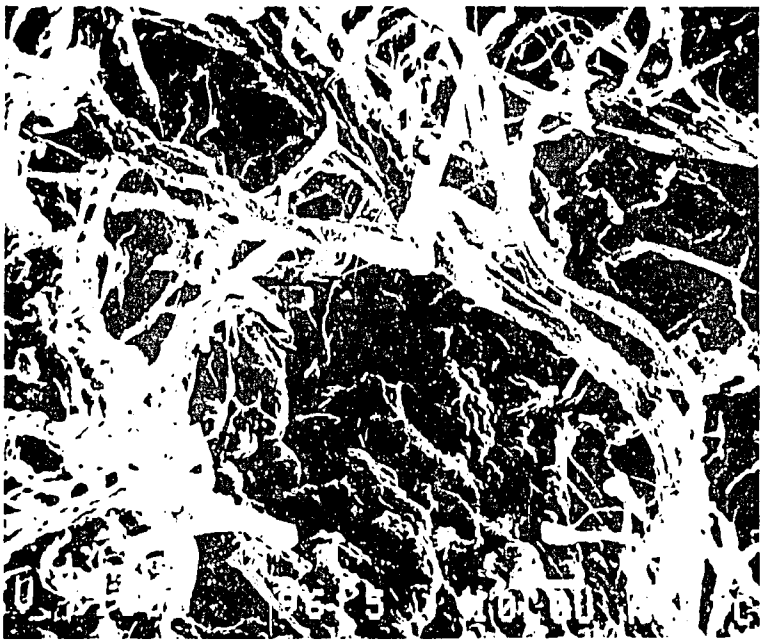

Fig. 3: Coffee pulp inoculated with *Pleurotus ostreatus* (F6)
(x 1000)

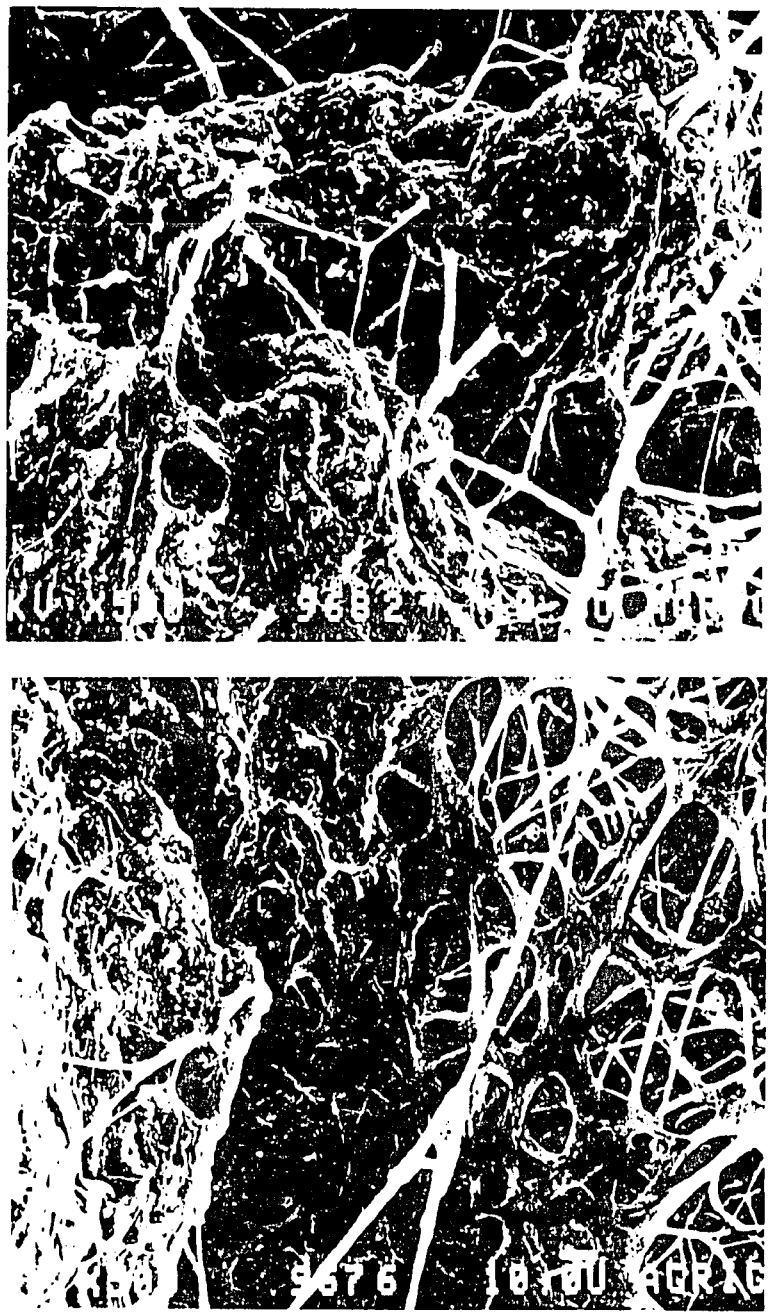

Fig. 4: Coffee pulp inoculated with *Schizophyllum commune* (x 500)

Fig. 5: Coffee pulp inoculated with *Schizophyllum commune* (x 1000)

B. Degrading enzymes produced in liquid culture

In this project we are studying lignin degradation by *Pleurotus*. However, the "standard" lignin degrader is the fungus *Phanerochaete chrysosporium*. Thus we have decided to compare the two fungi.

Pleurotus F6 and *Phanerochaete chrysosporium* BKM were grown in liquid culture with or without oxygen treatment. Under these conditions, *P. chrysosoporium* is known to produce ligninase, a major enzyme involved in the degradation of lignin. Indeed, we have found high activity of this enzyme produced by *P. chrysosporium* at levels similar to those described in the literature

Since our project is focussed on the edible mushroom, *Pleurotus*, we have studied fungal growth and enzyme production under the same conditions. Fungal biomass accumulation (Fig. 2), respiration (Fig. 3) and glucose concentration rates (Fig. 4), were much higher in fungus treated with oxygen during growth. On the other hand, no difference in protein excretion was observed (Fig. 5) which means that specific protein excretion (mg protein/mg biomass) was higher in the non-treated control. Enzyme activity was studied in the same cultures. No liginase activity was found when veratryl alcohol was used as a substrate. Several aromatic compounds were used as inducers but none were able to induce ligninase activity.

Laccase activity was also studied. High activity was found in cultures not treated with oxygen. Addition of oxygen decreased enzyme activity (Fig. 6). On the other hand, *P. chrysosporium* does not produce laccase.

It seems that enzymatic mechanisms of degradation of lignin are different between *P. chrysosporium* and *Pleurotus* F6. Since laccase activity alone is not enough to explain lignin degradation, we aim to

investigate this area further and include H_2O_2 producing enzymes and the role of free radicals. The results of this part of the research program will enable us to better understand lignin degradation and thus improve and support the practical part of upgrading lignocellulosic waste.

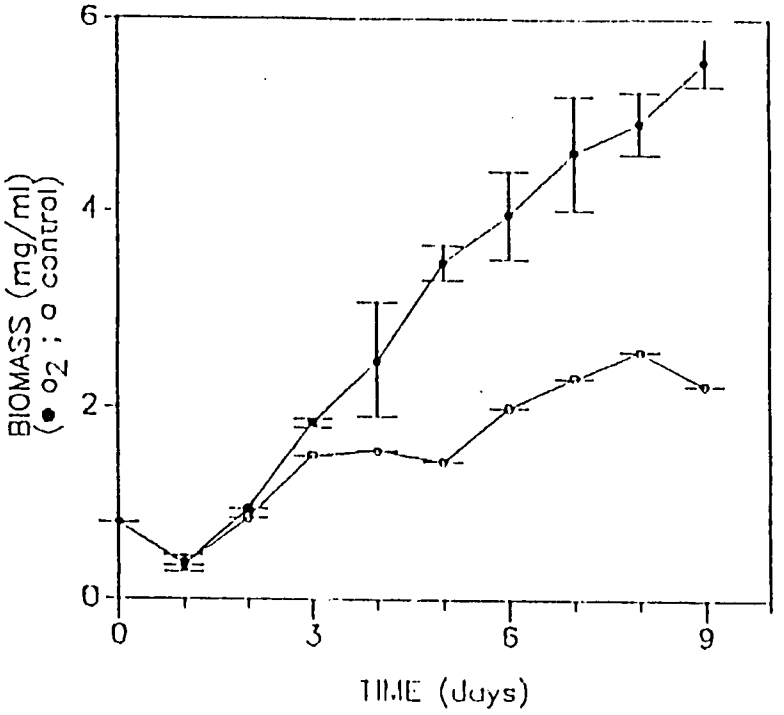

Fig. 2: *Pleurotus* biomass accumulation during growth in liquid culture

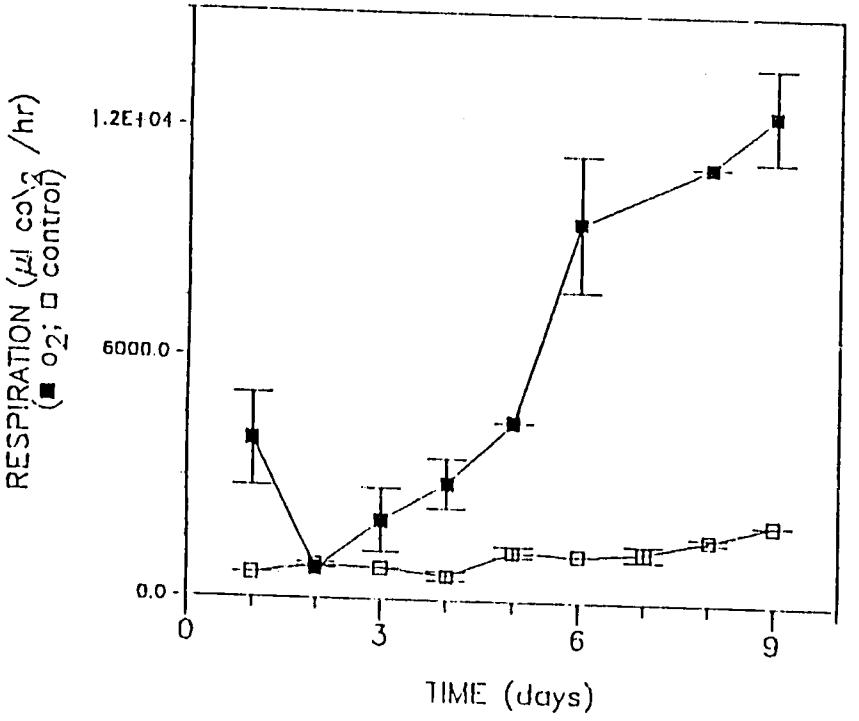

Fig. 3: *Pleurotus* respiration during growth in liquid culture

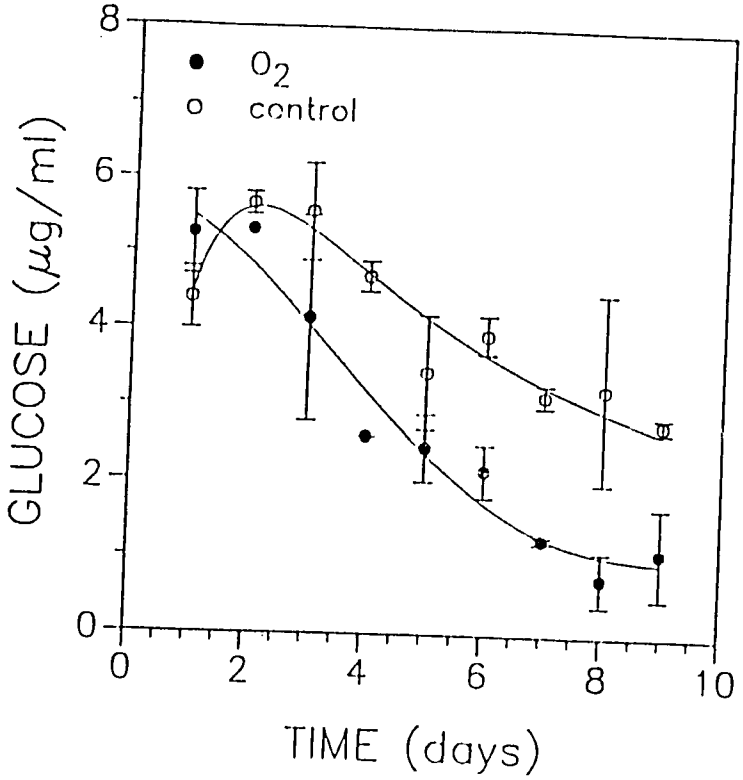

Fig. 4: Glucose consumption by *Pleurotus* grown in liquid culture

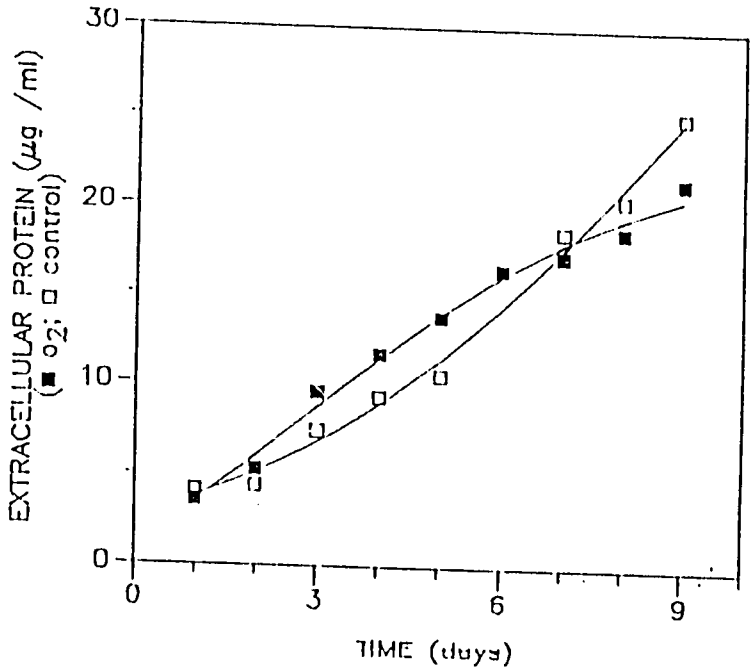

Fig. 5: Extracellular protein in *Pleurotus* liquid culture

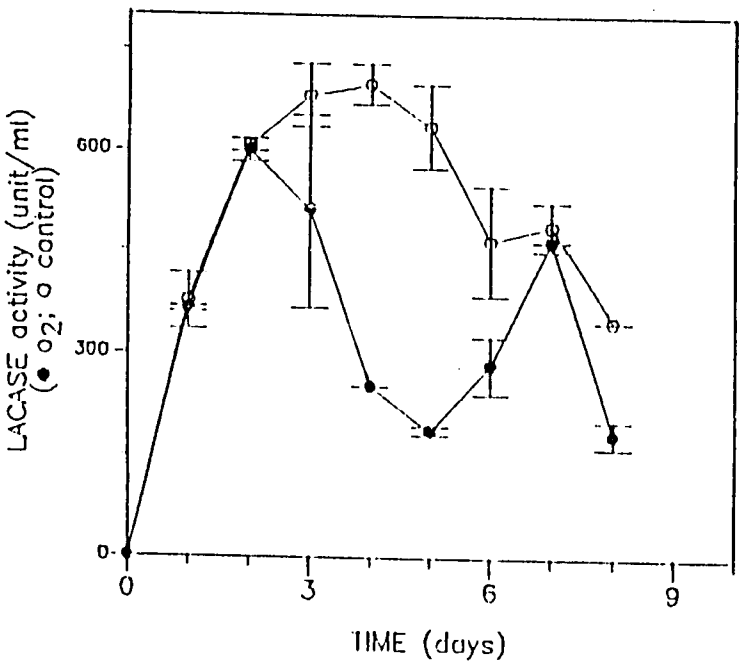

Fig. 6: Laccase activity produced by *Pleurotus* grown in liquid culture

C. Effect of O_2 and phenlics on several fungal activities

Pleurotus was grown in synthetic medium at 30°C for 4 and 7 days. Several activities related to aromatic compound degradtion was followed: Poly b decolorization (Fig. 7A); TMB oxidation (Fig. 7B); KTBA degradation to ethylene (Fig. 7C), and laccase activity (Fig. 7D). All activities were significance both with air and oxygen.

The effect of the addition of 2,6, dihidroy-benzoic acid (DHA) and veratyle alcohol (VA) to culture medium wa studied using the same parameters (Figs. 8A-D). Some activities were enhanced by VA but others were enhanced by DHA. It is concluded that *Pleurotus* is affected by these compounds and that such activities were probably connected to the phenolic compound degradation.

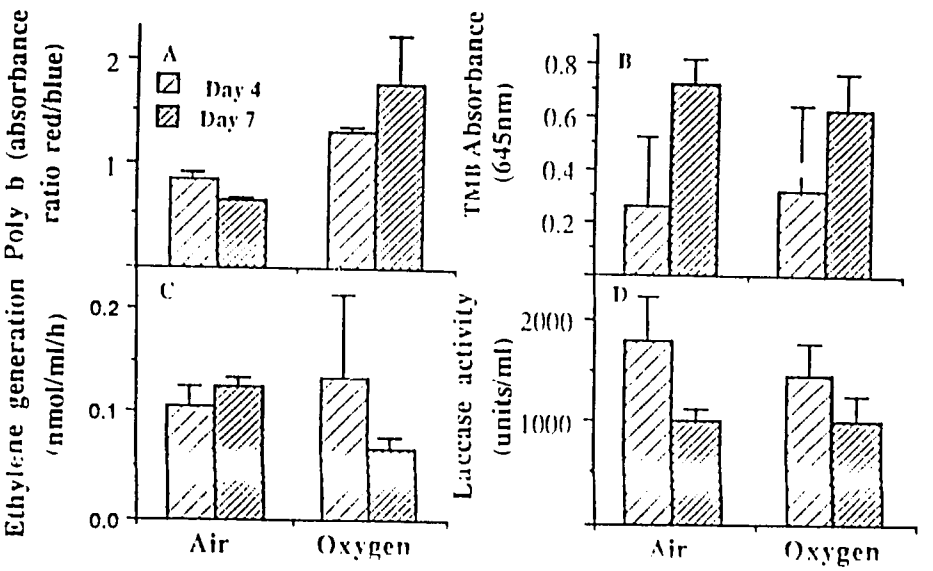

Fig. 7: The effect of atmospheric O_2 on *Pleurotus* activities

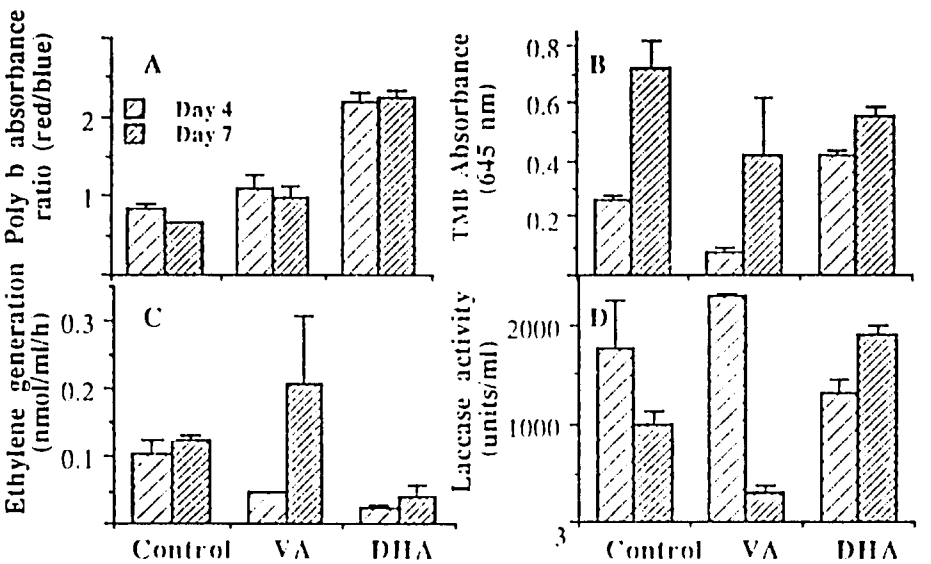

Fig. 8: The effect of phenolic inducers on *Pleurotus ostreatus*

11.7 Appendix 7.

"Final report of The Volcani Center (Israel)".

The Composition and In-vitro digestion of Coffee Pulp Products.

Final Scientific Report

Submitted to ICAITI-GUATEMALA

by:

D. BEN-GHEDALIA and J. MIRON

The Metabolic Unit

Agricultural Research Organization, ISRAEL.

February, 1993

INTRODUCTION

Coffee pulp is an important agro-industrial by-product in Guatemala and generally in coffee producing countries. Animal feeding appears to be the most economic target-use. However, some intrinsic factors hamper its use as an animal fodder; coffee pulp is high in lignin, phenolics and caffeine. Rumen microflora lacks the conditions and the enzymatic (oxidation) machinery required to cope with lignin, which is regarded as a major biodegradation obstacle. Phenolics and caffeine are known to exert detrimental effects both on rumen microflora and on the host animal. Therefore, a biological treatment of the pulp prior to feeding, aimed at reducing the level of the anti-nutritional factors, would be beneficial. Various strains of fungi were examined and screened by Y. Hadar and I. Chet from the Faculty of Agriculture, The Hebrew University, Israel, and the most successful ones which showed the highest growth rate on coffee pulp were found to be Pleurotus ostreatus (F6) and Schizophyllum commune, which were chosen for further compositional and in-vitro analyses performed in the Metabolic Unit.

The untreated and fungi-treated pulp samples underwent detailed chemical analyses and in-vitro digestion of the dry matter and carbohydrate constituents, and the results are reported herein.

MATERIALS AND METHODS

Analytical and in vitro digestion procedure

Freeze-dried untreated and fungal treated coffee pulp samples were ground to 1 mm by a hammer-mill and were used for the determination of neutral detergent fiber (NDF) and permanganate lignin as described by Van-Soest et al. (1991). Monosaccharides in coffee pulp and in the in vitro residues were determined in ball milled materials, after hydrolysis in 24N H_2SO_4 for 1h at room temperature, then in 1N-H_2SO_4 at 100°C for 5 h. Inositol was added as an interanl standard, and the released monosaccharides were determined as their alditol-acetate derivatives after reduction and acetylation according to Blakeney et al. (1983). The conditions for separation by gas-liquid chromatography were those of Bacon and Gordon (1980). Total N was determined by the Kjeldahl procedure. Organic matter was determined by ashing the samples in a muffle furnace at 600°C for 2h, and dry matter (DM) at 105°C. In vitro digestion test was done according to the rumen fluid-acid pepsin technique of Tilley & Terry (1963).

RESULTS

Chemical composition

The chemical composition of the pulp is shown in Table 1. A slight increase in protein content and soluble carbohydrates was noticed following the fungal treatment. lignin was hardly changed, however, cell-wall NGP which serves as the best estimate for true hemicellulose, was remarkably reduced by the action of the fungi. Monosaccharide composition is given in Table 2. The data of this table, clarifies those of Table 1. Generally, there is very little change in the composition of the monosaccharide constituents. The major effect of the fungi was expressed in solubilization of the hemicellulose constituents, and especially the potentially branching units: arabinose, galactose and uronic acids.

In vitro digestibility

The in vitro digestion results are given in Table 3. The in vitro test is a solubilization test, therefore, one should not be too much impressed by the high values of in vitro dry matter digestion (IVDMD) of the pulp. Some of the solubilized materials might prove indigestible in vivo. More important are the IVD values of the monosaccharide components. Glucose and xylose reside almost exclusively in the cell-wall as shown in Table 2. Therefore, IVD values of 75% for glucose (cellulose) and around 50% for xylose (xylan) are indicative of massive CW degradation. Those values are particularly high for a material containing 23% lignin in its dry material and ~46% lignin in its cell-walls. Coffee pulp is rich also in pectin (~10% on a DM basis) which is highly digestible (~95%).

Table 1. Chemical composition (g/100 g DM) of coffee pulp, untreated and after fungal treatments.

Component	Untreated	Fungal treatment	
		Pleurotus	Schizophyllum
Organic matter	90.3	89.3	89.1
Protein (Nx6.25)	11.6	12.7	12.6
ND-soluble carbohydrates	14.0	16.9	17.0
NDF	49.7	48.8	49.6
Hemicellulose (NDF-ADF)	2.40	2.00	5.20
Cellulose	23.1	22.1	20.0
Lignin (permanganate)	24.0	23.7	23.4
Cell Wall NGP	17.0	13.2	11.4

ND= Neutral detergent.

NDF = Neutral detergent fiber.

NGP= Non glucose polysaccharides.

Table 2. Composition (g/100 g DM) of monosaccharide residues, and their distribution (%) in cell-walls (CW) and cell solubles (CS) of coffee pulp, untreated and after fungal treatments.

				Fungal treatment					
	Untreated			Pleurotus			Schizophyllum		
Monosaccharide residue	Composition	Distribution In CW	Distribution In CS	Composition	Distribution In CW	Distribution In CS	Composition	Distribution In CW	Distribution In CS
Glucose	17.7	99.6	0.04	18.9	96.3	3.70	19.9	95.5	4.50
Xylose	3.76	92.7	7.30	3.43	94.3	5.70	3.30	93.0	7.00
Arabinose	8.45	75.3	24.70	7.86	52.3	47.7	8.03	46.5	43.5
Galactose	3.84	67.4	32.6	3.80	52.9	47.1	2.97	50.3	49.7
Mannose	3.27	75.5	24.5	3.83	59.4	40.6	3.59	54.3	45.7
Uronic acids	11.4	17.5	82.5	10.1	14.3	85.7	9.18	12.3	87.7
Total monosaccharides	48.7	71.3	28.7	48.2	65.0	35.0	47.4	64.2	35.8

CW = Cell walls ; CS = Cell solubles.

Table 3. *In vitro* digestion (%) of monosaccharide components of coffee pulp, untreated and after fungal treatments.

Monosaccharide Component	Untreated	Fungal treatment Pleurotus	Fungal treatment Schizophyllum
Glucose	76.8	73.4	73.8
Xylose	54.1	46.6	47.1
Arabinose	95.6	91.1	92.2
Galactose	91.0	88.0	87.1
Mannose	88.3	87.8	88.2
Uronic acids	95.3	94.0	92.9
Total monosaccharides	84.6	81.2	81.0
Dry matter	68.3	64.1	63.5

Arabinose, which is a major component of the soluble fraction is also highly digestible, as are galactose and mannose. Finally, the IVD of the total monosaccharide residues in coffee pulp was found to be high: 85%, and was affected very little by the fungal treatments. Coffee pulp has a remarkable potential as an energy-feed alternative. Therefore, the search for microorganisms able to degrade effectively lignin, caffeine and phenolics, should be continued.

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