

AGENCY FOR INTERNATIONAL DEVELOPMENT
R O C A P



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June 6, 1989

Mr. W. Ludwig Ingram, Jr.
Director a.i.
Instituto Centroamericano de Investigacion
y Tecnologia Industrial, I C A I T I
P.O.B. 1552
Guatemala, Guatemala C.A.

Subject: Research Proposal on "Biological Upgrading of Pretreated Coffee
Pulp". Grant funding authorized under Project No. 936-5544.21

Dear Mr. Ingram:

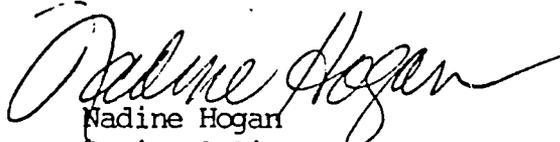
Pursuant to the authority contained in the Foreign Assistance Act of 1961, as amended, and the Federal Grant and Cooperative Agreement Act of 1977, the Agency for International Development (hereinafter referred to as "A.I.D." or "Grantor") hereby grants to Instituto Centroamericano de Investigacion y Tecnologia Industrial, (hereinafter referred to as "ICAITI" or "Grantee") the sum of \$200,000 to provide support for a research program entitled, "Biological Upgrading of Pretreated Coffee Pulp", as described in the Schedule of this Grant, Attachment 2 entitled "Program Description", and the Grantee's proposal, as amended, which is made a part of the grant and incorporated herein by reference.

This grant is effective, and obligation is made as of the date of this letter and shall apply to commitments made by the Grantee in furtherance of program objectives during the period beginning June 30, 1989 and ending June 30, 1993.

This grant is made to ICAITI on condition that the funds will be administered in accordance with the terms and conditions as set forth in Attachment 1 entitled, the "Schedule", Attachment 2 entitled "Program Description", and Attachment 3 entitled "Standard Provisions", which have been agreed to by your organization.

Please sign all copies of this letter to acknowledge your receipt and acceptance of this grant. Keep one copy for your files and return the original and all remaining copies, including all those stamped "Funds Available", to this office.

Sincerely,

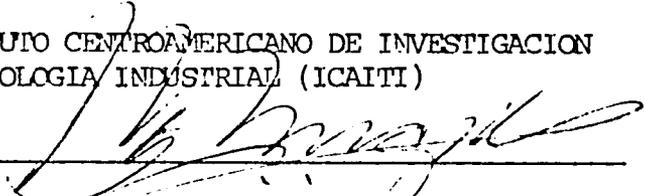

Nadine Hogan
Regional Director
ROCAP

Attachments:

1. Schedule
2. Program Description
3. Standard Provisions

ACKNOWLEDGED:

INSTITUTO CENTROAMERICANO DE INVESTIGACION
Y TECNOLOGIA INDUSTRIAL (ICAITI)

BY: 

TYPED NAME: W. Ludwig Ingram, Jr.

TITLE: Director

DATE: June 28, 1989

936-5544.21
ICAITI

FISCAL DATA

Appropriation:	72-1191021.6
Budget Plan Code:	DDSA-89-29520-KG11
Allotment:	948-51-520-00-19-91
Project No.:	936-5544.21
Total Estimated Amount:	\$200,000
Total Obligated Amount:	\$200,000
Funding Source:	AID/W
Project Office:	AID/SCI
Voucher Paying Office:	ROCAP
Proposal No.	C8-108

CABLE REFERENCE: STATE 171460, MAY 31, 1989

ATTACHMENT 1

SCHEDULE

A. Purpose of Grant

The purpose of this Grant is to provide support for the Grantee's program entitled "Biological Upgrading of Pretreated Coffee Pulp", as more specifically described in Attachment 2 to this Grant entitled "Program Description".

B. Period of Grant

The effective date of this Grant is June 30, 1989. The expiration date of this Grant is December 31, 1991.

C. Amount of Grant and Payment

1. A.I.D. hereby obligates the amount of \$200,000 for the purposes of this grant.
2. Payment shall be made to the Grantee in accordance with the procedures set forth in Attachment 3 - Optional Standard Provision No. 1 entitled "Payment - Periodic Advance".
3. All documentation required for payment under this provision shall be submitted to ROCAP Controller's Office, Guatemala City.

D. Grant Budget

The following is the Budget for this Grant, including local cost financing items, if authorized. Revisions to this Budget shall be made in accordance with Mandatory Standard Provision of this Grant entitled "Revision of Grant Budget".

6/30/89 - 6/30/93

<u>Line Item</u>	<u>ICAITI</u>	<u>Volcani Center</u>	<u>Hebrew Univer.</u>	<u>Total</u>
Salaries	43,000	9,600	9,400	62,000
Equipment	22,000	8,000	10,000	40,000
Mat. & Supplies	5,000	6,500	6,600	18,100
Travel & Per Diem	11,700	6,800	6,800	25,300
Overhead	24,700	7,700	8,200	40,600
Specific Contract (US of Costa Rica)	14,000	-	-	14,000
	=====	=====	=====	=====
Totals	120,400	38,600	41,000	200,000

Within the total amount obligated under the grant, the Grantee may adjust the line items within and between budgets as reasonably necessary to achieve the goals of the program.

E. Reporting and Evaluation

1. **Progress Reports:** Progress reports are required every six months. The principal investigator will submit progress reports stating what has been accomplished to date, including as an attachment the Financial Status Report, as discussed below. These reports are due within thirty days following the reporting periods ending June 30 and December 31 for each year of the grant. One copy of the progress report should be sent to the Science Attache, U.S. Embassy, Tel Aviv. Three copies of the progress report should be submitted to the U.S. - Israel CDR Program, Room 720 SA-18, Agency for International Development, Washington, D.C. 20523. Two copies should be submitted to A.I.D., PPC/CDIE, SA-18—Room 215, Washington, D.C. 20523 and two copies for ROCAP.
2. **Final Report:** The principal investigator will submit to the Office of the Science Advisor, Agency for International Development, Washington, D.C. 20523, a final report in three copies by June 30, 1993. At the same time, three copies of the final report will also be submitted to PPC/CDIE, Agency for International Development, Room 215 SA-18, Washington, D.C. 20523. One copy of the final report will be submitted to the Science Attache, American Embassy, Tel Aviv. The report should be sufficiently detailed to substantiate the findings and to permit a scientific evaluation of the research. Overseas collaborators shall be given fair credit for their participation in the research and a chance to review and comment on the final report before it is submitted. The principal investigator should share a draft of the final report with the U.S. - Israel CDR Program for comment prior to the formal submission. In order to promote dissemination, peer review and improvement of project results, the principal investigator(s) shall send copies of the draft final report to at least three recognized researchers in the field of the grant -- preferably in countries other than that of the investigator -- for comment prior to submission to A.I.D. Publication of the results in scientific journals is also encouraged.
3. The Financial Status Report (SF-269) is required every six months and will be submitted as an attachment to the progress report.
4. **Voucher Paying Office:** ROCAP

5. Principal Investigator:

Carlos Porres
Applied Research Division
Instituto Centroamericano de Investigación
y Tecnología Industrial (ICAITI)
Avenida La Reforma 4-47 zona 10
Guatemala, C.A.

6. Collaborating Institutions:

- The Hebrew University of Jerusalem
- The Faculty of Agriculture
- Rehovot, Israel

- Agricultural Research Organization
- The Volcani Center
- Bet Dagan, Israel

F. Special Provisions

1. Funding is contingent upon certification by appropriate institutional committee (in each country involved) that the research does not present unacceptable environmental hazard. The principal investigator should specify environmental safeguards and provide plans for containment to the U.S. - Israel CDR Programs.
2. While in the field, the Grantee will keep the A.I.D. field missions generally apprised of their work, but will not request administrative support except for the usual in-country introductions as may be appropriate. The Grantee will abide by Mission and host government regulations and customs as they apply to A.I.D. - supported in - country activities.
3. Project Officer approval required by paragraph (a) of Optional Standard Provision 3 of this grant entitled "Air Travel and Transportation" is hereby granted for travel described in the project description and included in the budget.
4. The principal and co-principal investigators of the Israeli and collaborating IDC institutions and essential scientific staff may not be changed during the life of the research without prior written approval of the U.S. - Israel CDR Program.
5. Compliance with Federal Guidelines and Regulatory Procedures: The Grantee shall implement this research activity, whenever it involves recombinant DNA, in accordance with:

- a. the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules;
- b. procedures issued by the USDA, EPA, or other appropriate Federal agency, regarding testing of genetically engineered organisms;
- c. A.I.D.'s environmental procedures; and
- d. such other Federal guidelines and procedures as may apply during the course of research.

Additionally, the Grantee cannot commence testing in any foreign location until written approval for such testing is obtained from A.I.D. and the government of the country where testing is planned. Testing shall be conducted in accordance with all applicable regulations of that country.

In addition, and prior to commencement of any such testing, the Grantee shall make a judgement and communicate the same to A.I.D. as to whether the regulations, procedures or facilities of the country in question are adequate to ensure testing in an environmentally sound manner. In the event such judgement is that they are not, the Grantee and A.I.D. will consult and agree on the conditions to be applied to the testing which will have such environmental effect.

Reports submitted under this Grant to A.I.D. will address regulatory issues as above related to the activity.

6. The Standard Provisions applicable to this grant are entitled "Mandatory Standard Provisions for Non-U.S. Nongovernmental Grantees" and "Optional Standard Provisions for Non-U.S., Nongovernmental Grantees".

G. Overhead Rate

Provisional indirect cost rates shall be established for each of the Grantee's accounting periods during the term of this grant. Pending establishment of final rates, the parties have agreed that provisional payments on account of allowable indirect costs shall be at the rates, on the bases, and for the periods set forth below:

<u>Type</u>	<u>Rate</u>	<u>Base</u>	<u>Period</u>
ICAITI-Overhead	30%	Direct Costs	6/30/89
Volcani			Until Amended
Overhead	25%	Direct Labor	6/30/89
HUT	25%	Direct Labor	Until Amended

NOTE: Notwithstanding Optional Standard Provision 16, the Grantee shall not be reimbursed for more than the indirect cost rates set forth above during the term of this grant.

H. Title to Property

The title of property acquired under this grant will vest in the Grantee in accordance with Optional Standard Provision No. 18 entitled "Title to and Use of Property (Grantee Title)". When the principal Grantee makes a subagreement under this grant agreement, the principal Grantee will pass to the subgrantee the provisions of the above cited reference as appropriate. Equipment purchased on behalf of each collaborating institution (LDC and Israel) shall remain the property of that institution.

I. Publications

Acknowledgement of A.I.D.'s contribution to any publication resulting from this grant shall be made in accordance with procedures set forth in Attachment 3 - Optional Standard Provision No. 10.

J. Closeout Procedures

This section prescribes uniform closeout procedures for this grant.

1. The following definitions shall apply for the purpose of this section:

- a. Closeout. The closeout of a grant is the process by which A.I.D. determines that all applicable administrative actions and all required work of the grant have been completed by the Grantee and A.I.D.
- b. Date of Completion. The date of completion is the date on which all work under the grant is completed, or the date on the award document, or any supplement or amendment thereto, on which A.I.D. sponsorship ends.
- c. Disallowed Costs. Disallowed costs are those charges to a grant that A.I.D. or its representative determines to be unallowable, in accordance with the applicable Federal cost principles or the conditions contained in the grant.

2. A.I.D. closeout procedures include the following requirements:

- a. Upon request, A.I.D. shall make prompt payments to a Grantee for allowable reimbursable costs under the grant being closed out.
- b. The Grantee shall immediately refund any balance of unobligated (unencumbered) cash that A.I.D. has advanced or paid and that is not authorized to be retained by the Grantee for use in other grants.

- c. A.I.D. shall obtain from the Grantee within 90 calendar days after the date of completion of the grant all financial, performance, and other reports required as the condition of the grant. A.I.D. may grant extensions when requested by the Grantee.
- d. When authorized by the grant, A.I.D. shall make a settlement for any upward or downward adjustments, to A.I.D.'s share of costs after these reports are received.
- e. The Grantee shall account for any property acquired with A.I.D. funds, or received from the Government in accordance with the provisions of OMB circular A-110, Attachment K.
- f. In the event a final audit has not been performed prior to the closeout of the grant, A.I.D. shall retain the right to recover an appropriate amount after fully considering the recommendations on questioned costs resulting from the final audit.

K. Source and Origin of Goods and Services

1. The country or countries where research or other scientific/technological cooperation takes place shall be deemed to be the cooperating country for the purpose of permitting local cost financing.
2. Goods and services, except for ocean shipping, financed by A.I.D. under the project shall have their source/origin in the Cooperating Country or in the United States as well as other Code 941 countries.
3. Ocean shipping financed by A.I.D. under the project shall, except as A.I.D. may otherwise agree in writing, be financed only on flag vessels of the United States.

ATTACHMENT 2

PROGRAM DESCRIPTION

A. Purpose of Grant

The purpose of this grant is to support the study of the upgrading of coffee pulp waste to an acceptable animal feed through bioconversion.

B. Specific Objectives

The investigators propose to select the best microorganism or mixture of microorganism which will upgrade coffee pulp waste to an acceptable animal feed by removing from it toxic compounds like the phenolics (chlorogenic acid and derivatives), lignin, the methylxantines (caffeine) and pigments, during their growth in solid state culture.

They also intend to find how does the digestibility of the holocellulose fraction of the pulp change with microbial growth. Finally, they want to optimize the operation of a pilot solid state fermenter operated in a repeated batch scheme.

C. Implementation

The following material is excerpted from the Grantee's proposal which is been incorporated as revised by reference.

1. TECHNICAL WORK PLAN

1.1. Technical Field on Involved Institutions

The institutions involved will contribute in the following scientific fields:

- a) The Hebrew University, Faculty of Agriculture, Rehovot: microbiology of fungal growth, substrate and product physico-chemical characterization.
- b) Agricultural Research Organization, The Volcani Center, Institute of Animal Science, Bet Dagan: in vitro ruminal digestibilities, substrate and product physico-chemical characterization, advice to be given for animal feeding trials.
- c) University of Costa Rica, Facultad de Agronomia, Escuela de Zootecnia: animal feeding trials.
- e) Central American Research Institute for Industry: microbiology and biotechnology of microorganisms growth, substrate pretreatments, substrate and product, physico-chemical characterization, pilot plant equipment design and operation; pilot plant production of processed products for animal feeding trials.

1.2 Description of Research Work

1.2.1. Coffee Pulp Pretreatments

Pretreatments will be done in order to enhance microbial growth, to induce beneficial changes in the lignocellulosic fraction and to reduce toxic factors. The following will be done: ensilage, mechanical pressing, steam cooking and ammoniation. The fresh and the pretreated samples will be analyzed for: caffeine, chlorogenic acids, caffeic and quinic acids, total polyphenolics, condensed and hydrolyzable tannins, proteic nitrogen, lignin, in vitro digestibility, particle size, surface area, pore size distribution and a record on electron microscopy.

ICAITI, The Hebrew University and the Volcani Center will work in developing some of the analytical techniques, specially the ones dealing with polyphenolic compounds and tannins. It is important to know if polyphenols are bound to caffeine, to protein, to holocellulose and to lignin; and if so how are these bonds modified during pretreatments. The objective of the pretreatments will be to break these bonds. It is also expected in the pretreatment that more surface area is created and pores are enlarged. If these two events happen a sharp increase in the in vitro ruminal digestibility should be observed.

Ensilage and mechanical pressing of the pulp are obligatory operations to be performed. Ensilage is needed in order to keep the pulp during the three months of the coffee season and process it during the nine off-season months. Pressing will eliminate the soluble part of the caffeine and polyphenolic compounds. Ammoniation will swell the lignocellulosic matrix, some hemicelluloses will be hydrolyzed and some bonds between hemicelluloses and lignin or polyphenolics might be broken. Inorganic nitrogen will be fixed in the holocellulose chain and later trials will look if it is biologically available. Steam cooking will favor hemicellulose hydrolysis and will increase pore size distribution and surface area. The pretreatment will be explored alone or in a sequence. The effect of temperature and time will be explored in experimental designs.

Pretreated samples will be dried to about 40% moisture (it is important not to dry them completely because the fiber structure might collapse and it will be impossible to detect any beneficial pretreatment effects on surface characteristics) and will be sent to Israel. ICAITI does not have the equipment to obtain surface area, pore size distribution and electron microscopy. All of these tests will be done in Israel.

From these tests we expect to answer the following: a) Are pretreatments justified according to what they accomplish and its cost? and b) if so, what sequence is the best.

1.2.2. Initial Microbial Screening

Once physically and/or chemically pretreated the pulp will be biologically modified by the action of microorganisms. The modifications that we are looking are: a decrease of free caffeine and polyphenolic compounds by their biological oxidations to CO₂ and H₂O or intermediate non toxic compounds and a decrease of lignin and the hydrolysis of the hemicellulose-lignin bond. If these two objectives are met and maximized we expect to have a much better response when fed to ruminants. We don't want to increase protein. Ruminants can synthesize most of it in the rumen. The ammonia added

during pretreatment could serve as nitrogen source for the ruminal fermentation. The original protein in coffee pulp should pass the rumen and be absorbed directly by the animal. It is important then to determine if there is any protein turnover during the biological pretreatment.

The biological testing will involve 26 white rot fungi, 7 filamentous fungi, 11 yeast strains and six aerobic bacteria. The yeast strains and the bacteria will be employed in mixed cultures only with either the white-rot and the filamentous fungi. The microorganisms are listed in Table 1.

The tests will be done in stationary glass jars at constant temperatures of 35°C, in solid state culture. The jars will be aerated for 15 minutes every day. We have obtained in the past good surface growth under these conditions in 2-3 days with filamentous fungi and 25-30 days for white rots (employing untreated, ensiled and pressed coffee pulp). However in this case we are interested in maximizing the degradation of caffeine, polyphenols and lignin. This might happen during active microbial growth or as a consequence of secondary metabolism, that is when there are serious starving conditions. In solid state culture of a substrate like coffee pulp this might be the case always. We do not know exactly what is limiting growth and when it is exhausted. So for each culture combination jars will be periodically analyzed and these changes monitored. The one (pure or mixed culture) which maximizes degradation is the one we will be looking for. We expect to find several which will compare and will select two or three at the most for further studies. The tests will be shared by ICAITI and the Faculty of Agriculture in Rehovot.

1.2.3. Pilot Plant Experiments

ICAITI will be in charge of designing a batch pilot reactor to treat 40-50 kg of wet pretreated pulp per batch. The reactor will be a cylindrical stainless steel vessel, having a perforated bottom where air will be forced in. It will also have a rotating blade to agitate the solids in the bed. Air will be circulated and its temperature, humidity and oxygen content will be monitored and controlled.

ICAITI will also design and construct a silo and pretreatment facilities. A rotating blade cylinder for steam cooking, ammoniation or both.

Tests will be done to optimize the batch operations. The minimum batch time to meet product changes will be determined for each microbial strain. Agitation and aeration schedules will be determined according to the specific biological demands of each microbe.

The resulting products will be used in the ruminal feeding tests either fresh or after partial dehydration.

1.2.4. Animal Feeding Tests

The animal feeding tests will be conducted under a specific contract with the University of Costa Rica, whose scientists will maintain a close exchange of results with those of the Volcani Center. Cattle of different ages will be employed. The maximum permissible amounts of untreated pressed coffee pulp have been already determined, as explained before, so that in these experiments up to twice the amount of pulp will be tested with the preated and biologically modified material. Daily weight gain, feed consumed and efficiency of feed conversion will be checked. Daily milk production will also be taken when applicable. In vivo dry and organic matter digestibility will be cross checked employing several animals.

Employing a smaller number of animals the effect of final moisture and storage time for the processed pulp will be explored. Material will be stored for up to 3 months employing intermediate moisture contents of around 30% and comparing it to a stabilized dried product of about 12% moisture. The moisture content is a critical process parameter as it bears heavily in the drying costs. The higher the moisture content the less expensive it is to process it.

TABLE 1. TENTATIVE LIST OF MICROORGANISMS

FILAMENTOUS FUNGI

Aspergillus niger (F-1024)
Aspergillus oryzae (F-1028)
Gliocadium deliquescens (F-1045)
Penicillium crustosum (F-1057)
Rhizopus oligosporus (F-1070)
Rhizopus oryzae (F-1072)
Trichoderma harzianum (F-1081)

The selected *Aspergillus* and *Rhizopus* species are employed in the manufacture of fermented human foods. Hence they do not produce microbial toxins and the quality of their protein is adequate. Their flavor and aroma generated in the fermented substrates is acceptable so that the palatability of the feed might be enhanced. *P. crustosum* degrades caffeine. *Gliocadium* and *Trichoderma* produce potent hydrolytic enzymes. We expect the fermented products to be quite different from each other.

WHITE ROT FUNGI

Agrocybe aergerita (F-1090)
Bondarzewia berkeleyi (F-1093)
Coriolus versicolor (F-1095)
Cyathus canna (F-1097)
Dichomitus squalens (F-1099)
Flammulina velutipes (F-1100)
Fomitopsis ulmaria (F-1101)
Ganoderma applanatum (F-1103)
Ischnoderma resinosum (F-1105)
Phebia radiata (F-1108)
Lentinus edodes (F-1105)
Phanerochaete chrysosporium (F-1118)
Polyporus anceps (F-1119)
Phanerochaete chrysosporium (F-1118)
Pycnoporus sanguineus (F-1112)
Pleurotus sajor-caju (F-1117)
Pleurotus flabellatus (F-1011)
Pleurotus ostreatus (F-1111)
Stropharia rugosoannulata (F-1116)
Trametes versicolor (F-1122)
Fomes fomentarius (F-1120)
Volvariella volvacea
Calvatia lilacinum
Tremella fuciformis
Agaricus bisporus
Auricularia auricula

The last five strains will be obtained from culture collections. Practically all of this white rots are potent holocellulolytic microorganisms and some of them have also true ligninases. They are the only microbes in nature which are able to grow in highly lignified tissues. Ligninase activity will be one parameter which will be assayed as a function of time during the screening experiments. We have preliminary information that they are able to degrade caffeine and polyphenolic compounds.

YEAST

Saccharomyces cerevisiae (L-131)
Saccharomyces ellipsoideus (L-106)
Saccharomyces uvarum (L-153)
Candida utilis (L-230)
Candida tropicalis (L-230)
Kluyveromyces fragilis (L-293)
Kloeckera sp (L-288)
Lypomyces kononenkokae (L-294)
Lypomyces starkeyi (L-296)
Pachysolen tannophilus (L-300)
Schawnniomyces castelly (L-314)

AEROBIC BACTERIA

Pseudomonas fluorescens var *cellulosa*
Cellulomonas uda
Thermomonospora curvata
Erwinia carotovora
Rhizobium phaseoli
Streptomyces viridosporus

Some of the yeasts show amylolytic and pectinolytic activities. The first three bacteria are highly cellulolytic, as is also the *Streptomyces*. *Erwinia* is active in enzymes degrading plant cell walls and *Rhizobium* towards plant roots. Selected combinations of the best five filamentous and best five white rots will be done with yeasts and bacteria. We are proposing to test this mixed cultures because there are previous references which show a positive effect of bacteria during the biological pretreatment of lignocellulosic residues employing fungi. Bacteria might be able to metabolize quickly some fungal inhibitors from the solid medium. The same comments apply to the yeasts.

The number in parenthesis identifies the culture in ICAITI's collection.

2.2 Technical Coordination Meetings

The key elements in the research programme proposed involve the quantification of the toxic factors in coffee pulp and their modification during the physical and biological treatments, and the structural changes of the lignocellulosic matrix.

This means that it is a prerequisite to be able to analytically quantify the amounts and the nature of the polyphenolic and tannin compounds in the pulp. To ascertain if they exist in a free state and/or bonded to other structural compounds.

Hence as the project first activity one ICAITI scientist will travel to Israel to meet with scientists of the Faculty of Agriculture at Rehovot and the Volcani Center. Taking as a base a literature review made in advance an experimental protocol will be made for developing a series of analytical methods to solve the above mentioned problem. We foresee a series of tests that will be done by Israeli scientists as we don't have the analytical equipment at ICAITI. Once this objective is met ICAITI scientist will return. The teams will start the development work and only when satisfactory experimental data is obtained will the programme continue.

Two central American students will participate in the initial microbial screening activities.

These first two activities are expected to end in 18 months. Immediately after, another coordination meeting will take place in Israel. This time ICAITI's principal investigator will make the trip. A detailed experimental plan will be elaborated for the pilot production of the improved pulp and the complete animal feeding test programme.

A final coordination meeting will take place in Central America at ICAITI. The principal investigators from the Faculty of Agriculture, Rehovot and The Volcani Center will visit the area. They will review the results from the animal feeding programme in progress and from the pulp production trials.

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10