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WORKING MATERIAL

CONSULTANTS GROUP MEETING ON

DEVELOPMENT OF COST-EFFECTIVE DIETS FOR USE IN MASS PRODUCTION OF TSETSE FLIES

Report and Recommendations of a Consultants' Group Meeting organized by the Joint FAO/IAEA Division of Nuclear Applications Food and Agriculture, Vienna, Austria, 17-21. 7.2000.

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CONTENTS

1. Executive Summary

The increasing demand for employing tsetse SIT for area-wide tsetse and trypanosomosis management programmes on mainland Africa has compelled the IAEA to concentrate on the development of semi-automated processes for standardising laborious and quality sensitive components of the sterile male mass production. The size of facilities required to produce the sterile males will continue to increase with time and demand. The current diet for tsetse is decontaminated vertebrate blood and it will need to be supplied to centres without access to a suitable local blood source.

In view of the increasing demand for sterile male tsetse and uncertainty of obtaining high quality decontaminated blood locally, ways need to be explored to ensure availability of inexpensive, standard quality diets. Towards this goal a consultants group meeting on the development of cost-effective diets for tsetse was held at the IAEA headquarters in Vienna, Austria from 17 to 21 July 2000. The major objective of the consultants group meeting was to identify research that is needed to ensure the availability of large quantities of high quality diet for tsetse mass production.

Seven papers were presented and discussed. A visit was made to the Entomology Unit, at the FAO/IAEA Agriculture and Biotechnology Laboratories, Seibersdorf to see the present tsetse rearing facility and the various steps of blood processing and quality assurance used in the evaluation of blood quality before use for colony feeding.

The meeting noted that commercially available products are used to prepare standard diets for screwworm mass production. These products have not yet been adequately evaluated for tsetse. However, it is necessary to improve the current procedure applied to the use fresh blood. Possibilities of utilising commercially available dietary ingredients should also be explored.

A three-step approach was proposed:

- Improvement and optimisation of the current blood collection, processing etc.
- Use of additives for enhancing processing and increasing the nutritional value of processed diets.
- Development of artificial diets to reduce/eliminate dependence on fresh blood.

Activities/investigations that should be undertaken were identified and potential collaborating centres suggested. Options for privatisation was also considered.

2. Background

Mass production of millions of insects daily is a vital part of any Sterile Insect Technique (SIT) programme. Following successful colonisation of tsetse using the membrane feeding technique, mass rearing of this vector became a reality.

As a result of the successful eradication of tsetse flies from the Island of Zanzibar, other eradication programmes are being planned employing large scale SIT on mainland Africa. For example, in Ethiopia, it is anticipated to establish a membrane fed colony of 10 million tsetse females that would produce about 1 million sterile males for weekly aerial release. The membrane feeding technique will require 300 - 400l of quality tested blood daily. For this, about 60 - 80 cattle will need to be slaughtered per day. In Addis Ababa, where 400 cattle are being slaughtered per day, a convenient and acceptable procedure for collecting, processing, decontaminating, testing and storing blood will be needed.

However, the increased demand for tsetse SIT will necessitate that production facilities be sited in the region that may lack a suitable local blood source.

To develop an effective diet it is necessary to understand the nutritional requirements of tsetse in relation to reproductive performance, longevity and flight. These studies may also play an important role in lowering the cost of production.

In the mass production of screwworm commercially available products are used to prepare standard diets for larvae and adult flies. In order to achieve similar progress for tsetse mass rearing, studies will be needed to improve the present diet collection, processing, sterilising and storing, as well as the use of commercially available dietary ingredients to replace fresh blood.

Solving of the above problems should be approached in a three-phase plan:

1. Improve and further optimise fresh blood collection, processing and decontamination.
2. Evaluate the use of additives for processed blood.
3. Develop artificial diets.

3. Objectives of the consultants meeting

The terms of reference for the meeting were as follows:

1. Review the current method of blood collection and processing and to outline simple alternative methods of processing blood including decontamination and storage.
2. Make suggestions on commercially available components that may be useful to develop a standard and high quality, but simple and inexpensive blood diet.
3. Identify research needs and suggest priority issues to be addressed, either in Seibersdorf or through technical contracts.
4. Suggest names of possible collaborators who could assist in identified priority issues.

4. Summary of presentations

4.1 Current method of blood collection, processing and storage

Blood is obtained from abattoirs in or near Vienna. Blood is collected from bolt- shot bulls or from pigs after they are hung and their throats cut. A large funnel connected through a silicone hose to a 20l container is held at the throat of the animal. The blood flows into the container where it is defibrinated by stirring. Collected blood is poured through a milk sieve into a larger (500l) tank. The blood is then transferred into 5l cans and transported to the cool house where it is stored frozen at – 20° C until further processing and use. One 5l can out of each lot is brought to the laboratory to check quality control which comprise of bacteriological screening at different stages of processing and a bioassay. A visit to Seibersdorf provided the opportunity to see the present facility for tsetse rearing. The various steps of blood processing, rearing and feeding of tsetse flies were demonstrated.

4.2 Insect diet and reproductive physiology

Feeding and reproduction are the two most important activities of an insect's life. Research in insect's food consumption, utilisation and allocation not only allows us to understand specific selection pressures on these components, but also provides us with necessary tools to develop insect diets depending on the nutritional requirements of the insect.

Tsetse fly is an obligatory blood feeder possessing adenotrophic viviparous mode of reproduction, producing only one full-grown larva per reproductive cycle. More than 90 %

of dry weight of blood ingested by tsetse is protein or protein derivatives, there being very little lipids or carbohydrates. Therefore, the insect's metabolism is largely based on amino acid utilisation.

Demand for protein by the tsetse reproductive processes is not like normal egg laying insects. Because a pregnant tsetse has to provide nourishment for the developing larva in the uterus, most of the dietary proteins are diverted to synthesise milk in the uterine gland. A total blood meal of about 250 mg needs to contain all that is necessary to account for the composition of a full grown third instar larva.

4.3 Industrial processing of animal blood

Originally, blood from slaughterhouses was discarded leading to environmental problems because of its high level of organic material. American Protein Corporation (APC) now uses this blood and transforms it into products with a high value.

After an official *ante mortem* inspection, blood is collected into collection pans where anticoagulant is added. Blood from different animals is mixed and refrigerated, and transported to the processing plant in an isothermal tank. In the production plant, blood is processed, obtaining a wide range of products from blood, plasma, red cells and proteins.

To guarantee the quality, blood and products are subjected to quality and R&D controls from the time blood arrive at APC plant to when the product is with the customer. This quality control includes chemical, physical, microbiological and characterisation analyses.

4.4 Heat deactivation of viruses in whole blood

Various techniques exist for inactivation of infectious agents in plasma derivatives. Several steps in the fractionation and purification processes inactivate, remove or reduce in number, those micro-organisms that may have contaminated the plasma. Bacteria and large micro-organism can be removed by filtration, but viruses can not be removed by this process. The measures used for viruses involve treatment either with heat or with combination of solvents and detergents. Many manufacturers now use both heat and solvent-detergent treatments to decrease the risk of contamination of the final product.

4.5 Analysis of freeze-dried blood

Blood is a very heterogeneous mixture of cellular and plasma components. Used as a starting material one has to bear in mind that blood from different animal species differs quantitatively and/or qualitatively in a number of constituents. The nutritional value of blood products varies therefore in respect of its suitability for rearing tsetse flies.

Technological procedures like freeze-drying may have an impact on physical, chemical and biological aspects of the products. Problems may arise from the decrease in solubility coming from protein precipitation/aggregation phenomena, degradation or production of derivatives and inactivation of biologically active substances. Quality testing in comparison to reference material can be performed with chromatographic, electrophoretic, spectrophotometric, immunochemical and biological methods. Although analysis of most components is possible the nutritional value is still the main quality criterion.

Defining ingredients for a high quality rearing diet will require the establishment of a database from fresh or processed blood from different species, mixtures of different preparations, and artificial diets that have been tested, fed to the different *Glossina* species.

This matrix should allow the development of relevant logistics for finding adequate diet constituents for the different fly species.

4.6 Artificial diets for tsetse rearing, local blood sources, availability and logistics

The development of *in vitro* membrane feeding technique has facilitated studies in which the nutritional requirements of tsetse can be studied. Using this technique, a semi-defined synthetic diet for tsetse was developed. This diet consisted of commercially available ingredients and used to rear successfully *Glossina palpalis palpalis* for five generations.

To ensure and standardize blood used for tsetse mass rearing during the Zanzibar eradication project, all blood was collected in Vienna, screened and quality tested before shipment. The shipment of blood from Vienna, Austria to supply tsetse mass-rearing centres in Africa is not only costly and fraught with delays and logistical problems, it is also subject to quarantine restrictions and complicated by safety regulations governing the importation of labile biological materials from countries where contagious diseases are reported. Further difficulties are shortage of modern abattoirs, radiation source for decontamination and the requirement for a colony for bioassay of every batch of blood before shipment to a rearing centre.

4.7 Principles of tsetse diet quality control

For routine tsetse feeding on membrane, quality assurance of the blood is essential. Therefore, it is necessary to screen each batch of collected blood by conducting a 25 day feeding test with a small group of female tsetse. Results of such quality control test will provide information to calculate a quality factor (QF) which in turn is used as the criterion to decide whether or not the tested diet is suitable for mass rearing. The QF expresses the probability of tsetse colony females surviving and producing well developed larva at the end of a 25 days period. Several parameters are taken into account and used for calculating the QF and the minimum accepted level is set at 1.0.

5. Conclusions

1. Since both adult female tsetse and the larva within the uterus depend on the same source of blood, it follows that availability of blood is among the most important factors affecting reproductive physiology of tsetse in the colony and the quality of sterile males released in SIT campaigns. The present source of tsetse diet is decontaminated quality tested bovine blood and at times supplemented with pig blood for special needs of some species.
2. In the foreseeable future the demand for tsetse SIT component and availability of sufficient sterile males will increase and therefore, the number of facilities for mass production will be increased.
3. The current Seibersdorf method of fresh blood collection and processing is a well defined and working procedure for the current state of tsetse production.
4. Gamma irradiation as a means of blood decontamination restricts/limits tsetse mass rearing to centres that have access to an irradiation source or decontaminated blood.
5. In the affected countries of Africa, only Zambia and Republic of South Africa have facilities to meet some of the requirements for production of quality blood.
6. Procedures for blood processing like freeze-drying may have an impact on physical, chemical and biological aspects of the so processed product by reducing its solubility and degradation of biologically active substances. Current processing of

lypholising and reconstitution of bovine blood resulted in a diet that was found inadequate for supporting reproduction in tsetse.

7. A semi-defined artificial diet for tsetse consisting of commercially available ingredients was developed and used to rear *G. palpalis palpalis* for five generations.
8. Blood composition is known to vary among individuals of the same species and between species under the influence of sex, age and physiology and therefore important to ensure a reliable quantity of high quality diet supply to tsetse mass production facilities.

6.PROPOSED ACTIVITIES

Proposed activities are tabulated under major activities which were identified during the meeting.

A. FRESH BLOOD

Collection of fresh blood is limited by accessibility to abattoirs and unreliable source of suitable and adequate number of animals to be slaughtered.

Collection Procedure	Advantages	Disadvantages	Recommended Activity
Blood collection from bolt shot bulls and pigs after throat is cut, defibrination by stirring	• Rapid	• High level of hygiene required.	• Install closed system where the situation allows
Closed system using hollow needle from bolt shot bulls and pigs	• Minimum contamination	• Slow • Needs anticoagulants/ additives to avoid clotting	
Bleeding under 'non European-like' conditions (bleeding on the floor)	• Common procedure in Africa	• High risk of contamination • Less blood per animal collected	• Adapt slaughtering to European-like conditions

B. PROCESSING OF BLOOD AND TRANSPORT TO COLLECTING CENTER

Procedure	Advantages	Disadvantages	Recommended Activity
Defibrinated blood poured through a sieve into a large 500l tank, then distributed into 5l cans for transport to cool house and stored at –20°C	• Feasibility proven • No sophisticated equipment required	• Collection lasts several hours and may lead to deterioration in quality • Environmental conditions (temperature) may affect quality of blood • Large volume of blood (500l) difficult to obtain at one collection	• Using refrigerated transportable container with provision for defibrination

C. DECONTAMINATION

Procedure	Advantages	Disadvantages	Recommended Activity
1KGy gamma radiation	• Effective	• Limited to availability of irradiation source	• None
Ultra Violet radiation	• No need for irradiation source	• Appropriate equipment for high input to be designed and tested	• Evaluate use of UV radiation • Determine the efficacy of UV decontamination on product quality
Pasteurization /UHT treatment	• No need for irradiation source	• Appropriate equipment to be designed or adapted • Coagulation and or denaturation of proteins	• Evaluate pasteurization/UHT treatment in the presence of stabilizers • Determine the efficacy of method and effect on product

D. ALTERNATIVE TO FRESH BLOOD

Product	Advantages	Disadvantages	Recommended Activity
Freeze dried blood [FDB]	• Satisfactory diet for tsetse when FCS and BSA added • Good results when sonicated 1:1 mixture of porcine and bovine blood • Easy to transport and store	• Limited supply	• Test different additives, and proteins as well as different blood types as supplements to FDB • Analyze and identify the missing components in FDB
Spray dried blood [SDB]	• Commercially available • Easy to transport and store	• Suitability as tsetse diet unknown • Ash and insoluble content (<10%) • Highly irradiated and may affect quality	• Test SDB itself and after centrifugation for fly feeding • Test suitability in the presence of additives(FCS, BSA, fresh blood) • Characterize SPB and compare against FDB
Fractionated animal blood cells (RBC); used in feed industry as protein source, in building industry, as fertilizer, in insect diets and for clarifying wine	• Commercially available • Proven as protein source for insect diet (Screwworm)	• Suitability as tsetse diet component unknown	• Test role played by RBC in tsetse diet/ as additives • Evaluate different components as diet additives

E. ARTIFICIAL DIET

Product	**Advantages**	**Disadvantages**	**Recommended Activity**
KT 80: a semi-defined synthetic diet used to rear *Glossina palpalis palpalis* for five generations	• Chemically defined • 2.Simple to store ingredients with potential for standardization • Baseline information available for further research	• Cost –effective, suitable dietary requirements not defined • 2. Long term research needed	• Further test KT80 to improve composition • 2. Explore possibility of using cost effective ingredients of non blood origin

7.Privatisation

Privatisation may be perceived at several levels depending on the overall logistic and the investment capital.

The whole process could be contracted out to a private company that would undertake:

- Blood collecting and processing.
- Decontamination.
- Quality control.
- Storage and timely shipment of product.

The private company should control the:

1. Abattoir to ensure standard slaughtering and therefore high quality blood.
2. Cooled storage facilities on the abattoir.
3. Trucks with cooled tanks.
4. Storage plant.
5. Irradiation source or equivalent system for effective decontamination.

The production of tsetse diet alone may lead to economic risks and these may be minimised by producing high quality raw material (whole blood or defined fractions thereof) in addition to tsetse diet, or developing artificial diets sold to all tsetse mass-rearing centres.

To make it an attractive venture/investment with a guarantee for accelerated return of capital, a "Technological Centre" where high value blood products or new products depending on blood constituents could be designed and developed near or adjacent to the abattoir.

8.Collaborators

- American Protein Corporation (APC)- provision of blood derivatives, proteins and advise on blood processing
- Baxter-Austria – provision of Albumin and albumin derivatives.
- Bristol University – analyses of residues in animal tissues.
- Bodenkultur University (Meat technology) – analyses of residues in animal tissues.
- Iowa State University, Vet School – blood derivatives, proteins, blood processing technology.
- Technical University of Vienna - heat exchange, pilot systems for pasteurisation and UHT sterilisation.
- Vet Med University Vienna - purification and identification of blood constituents, peptide and protein analyses etc.
- USDA – test products with other haematophagus insects, product analyses.
- Tsetse rearing Centres in Africa – verification of procedures and establishing biological base line data on locally available blood.
- Universities, institutes and laboratories in Africa – verification of procedures and establishing chemical and residues baseline data on locally available blood

9.Recommendations

1. Explore possibility of installing a "closed system" of blood collection to further reduce contamination.
2. Evaluate the use of a transportable container with a stirrer and refrigeration system.
3. Investigate the effect of diluting blood with physiological saline on ingestion and digestion and pupal weight.
4. Evaluate the use of spray-dried blood and freeze dried blood and additives for colony maintenance.
5. Investigate alternative methods of decontamination including UV radiation and pasteurisation/ UHT procedures.
6. Investigate the possibility of developing a blood supply and reference centre in Africa for example in Zambia or Republic of South Africa.
7. Investigate use of additives for preventing coagulation at collection and during processing.
8. Re-evaluate the use of the semi-defined synthetic diet KT80

10.Acknowledgements

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11. Appendix I

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12. Appendix 2

CONSULTANTS' GROUP MEETING ON

Development of Cost Effective Diets for Use in Mass production of Tsetse Flies

17-21 July 2000
Vienna International Center, IAEA- Building A, Room 2210

Provisional Agenda

Monday, 17 July

09:00 Official Opening
09:15 Introduction Mr. Hendrichs
09:30 Administrative matters Ms. Opiyo
10:00 *Break*
10:30 The FAO/IAEA Tsetse Programme Mr. Feldmann
10:50 Entomology Laboratory programme Mr. Robinson
11:10 Current method of blood collection, processing, storage and use
Mr. Luger/Mr. Kabayo
11:40 Departure for Seibersdorf and *Lunch* on the way
13:45 Courtesy call on Head, Agriculture and Biotechnology Laboratory
14:00 Visit to the Entomology Unit Mr. Robinson
Demonstration of feeding, blood processing Mr. Luger/Mr. Frölich
View tsetse rearing in general
16:00 *Break*
16:30 Discussion to review the current methods of blood collection, processing and storage
18:00 Depart for Vienna

End of day one

Tuesday, 18 July

Presentation of working papers
09:00 Insect diet and reproductive physiology. Dr. Chaudhury.
09:45 Industrial processing of Animal blood. Dr. Ródenas
10:30 *Break*
11:00 Heat deactivation of viruses in whole blood. Mr. Atger
11:45 Analysis of freeze-dried blood- Dr. Gemeiner
12:15 Artificial diets for tsetse rearing, local blood sources, availability and logistics Mr. Kabayo
13:00 *Lunch*
14:30 Principles of tsetse diet Quality Control Ms Opiyo
15:45 Artificial diets for tsetse rearing, local blood sources, availability and logistics Mr. Kabayo

15:30 *Break*
16:00 Outline of key topics for discussion on Wednesday
17:00 *Cocktails*
End of Day two
.

Wednesday, 19 July

Discussions on Key Topics
09:00 Diet alternatives including utilisation of commercially available components
09:50 Alternative diet processing
10:40 Break
11:10 Alternatives for diet decontamination
11:50 Alternatives for diet storage
12:30 *Lunch*
14:00 Diet Presentation
14:40 Diet quality assurance
15:30 Break
16:00 Options for privatisation
16:30 Outline of proposed steps
17:00 *End of day three*

Thursday, 20 July

09:00 Report writing
- Proposed steps/phases of research/action, institutes involved and budget outline
- Commercial diet alternatives
- Diet processing
- Diet decontamination

12:00 *Lunch*
14:00 Report writing
- Diet storage
- Quality assurance
- Privatisation

17:00 *End of day four*

Friday, 21 July

09:00 Discussion of report
13:00 *Lunch*
15:00 Presentation of Conclusions and Recommendations
16:00 Closing
End of meeting