
C005148

Title

**Transgenic rice (LL Rice 62)
Feeding values of Transgenic rice (Event 62) in poultry-broilers**

Code

Authors

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Test guideline

Completed on

26 AUGUST 1999

Testing Facility

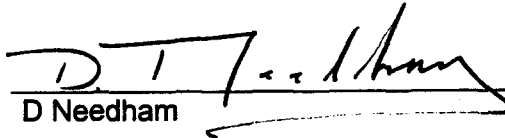
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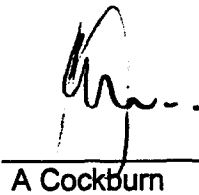
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C005148**APPROVALS PAGE**

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Head of Testing Facility


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Date (ddMONyyyy)

C005148

TNO report
V99.034 final

Feeding value of Transgenic rice (Event Rice 62) in Poultry-Broilers

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Project number:

20505

Study number:

805

Study code sponsor:

TOX 98232

Sponsor:

AgrEvo UK limited, Essex, England

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Number of pages:

31

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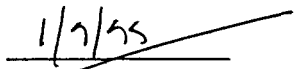
Approval

Sponsor

AgrEvo UK Limited

Dr C. Reynolds
(Study monitor)


Signature

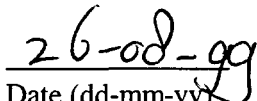

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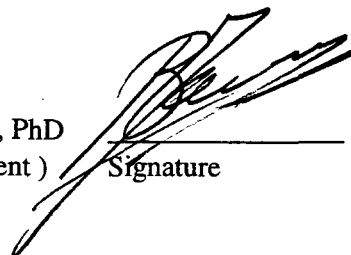
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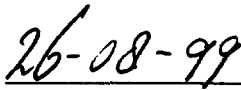
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Date (dd-mm-yy)

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List of abbreviations

bw	Body weight
DHDV	Human and Animal Nutrition Division
GM	Genetically modified
GMO	Genetically modified organisms
I&R	Identification and Registration number
SOP	Standard operating procedure

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1. Summary

To investigate the nutritional equivalence of the test products GM (Genetically modified) rice Event 62 (LLRice 62) and its non transgenic counterpart (non-GM) rice in broiler chickens, the zootechnical performance, general health and slaughter quality was determined in two groups of 60 male broiler chickens. Each group was subdivided into three replicates (=pens) of 20 broilers each .

The control group, group A, received diets with 30% non-GM rice. The test group, group B, received diets containing 30% GM rice Event 62.

During the in-life phase the following parameters were recorded:

- the behaviour and the physical condition of the birds, observed twice daily by general health inspection.
- individual body weight, determined at Day 7, 14, 21, 26, 35 and 42.
- at Day 0, after allocation, total body weight per pen was determined within two hours after arrival.
- mortality rate, the number of deaths per pen was recorded daily.
- feed intake, determined per pen at each time of weighing.

From body weight and feed intake, feed conversion efficiency was calculated.

The birds were slaughtered at Day 42, the parameters of interest were:

- carcass weight incl. abdominal fat
- breast muscle weight incl. bone excl. skin
- abdominal fat weight

Clinical signs and macroscopic findings were recorded by a pathologist.

To determine the nutritional equivalence of the two test products the data of two test groups were analysed statistically using the GLM procedure from SAS for the individual body weight and slaughter weights and a T-test for the pen related variables like feed intake.

No significant ($P > 0.05$) differences were found in feed intake, feed conversion efficiency, weight gain or slaughter quality between the the non-GM rice and the GM-rice Event 62.

The results demonstrate that the GM rice Event 62 (LL Rice 62) was nutritional equivalence to non-GM rice.

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2. Responsible personnel and testing facilities

2.1 Sponsor

Sponsor:
AgrEvo UK Limited
Chesterford Park
Saffron Walden
Essex CB10 1XL, England, UK
Phone: + 44 (0)1799 530123
Fax: + 44 (0)1799 573546

Study monitor :
Dr. C. Reynolds
Phone: +44 (0) 1799 573527
Fax: + 44 (0) 1799 573546
email: caroline.reynolds@agrevo.com

2.2 Testing facilities

The study was performed at:

TNO Nutrition and Food Research Institute
Department of ILOB
Haarweg 8
P.O. Box 15
6700 AA Wageningen
Phone:+31 317 499 440
Fax: +31 317 499 463
e-mail: schat@voeding.tno.nl

2.3 Responsibilities

The sponsor was responsible for the supply of sufficient test product.

Study director : B. Schat DVM BSc (1)
Phone: + 31 317 499 482
Email:schat@voeding.tno.nl

Deputy study director : G.M. Beelen BSc (1)
Phone: + 31 317 499 410
Email:beelen@voeding.tno.nl

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- Responsible for animal care : K. Deuring, D. van Kleef, J. van Harn, J. de Jong, A. Hoek. (1)
- Responsible for diet preparation/ feedmill : P.C. Roeleveld, C. Siebers
- Responsible for slaughtering : J. van Harn (1)
- Responsible for veterinarian investigation : Ms. B. Schat DVM BSc, B.P.M. Janszen DVM PhD (1), H.A.M. van Elst DVM, E.J. Kiemeneij DVM (3)
- Responsible for statistics : J. Wiebenga BSc (1)
- Responsible for pathology : M.V.W Wijnands DVM (2)
- Responsible for analysis : analyse Energy: Department of ILOB (1)
analyse Content : Pre-Mervo (4)
- Management Department : B.P.M. Janszen DVM PhD (1)

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Department of ILOB

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Phone :+ 31 30 69 44 487

(3) Veterinary Clinical Centre
'Kortenoord', Wageningen
Phone : +31 317 412432

(4) Pre-Mervo
Protonweg 10, Utrecht

2.4 Subcontracting

The analyses of the diets (dry matter, crude protein, ash, crude fat, Calcium and Phosphorus) were subcontracted to Pre-Mervo, Protonweg 10, Utrecht, the Netherlands, who are working under 'Sterlab'.

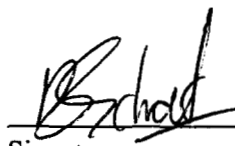
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3. Statement of GLP compliance

We, the undersigned, hereby declare that this report constitutes a true and complete representation of the procedures followed and of the results obtained in this study by the Division 3 of the TNO Nutrition and Food Research Institute. This study was carried out in accordance with the OECD Principles of Good Laboratory Practice (1997). Organization for Co-operation and Development (OECD), Paris. ENV/MC/CHEM(89)17.

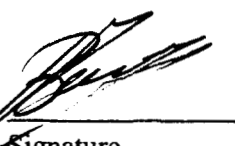
Submitted by:

Ms. B. Schat, DVM BSc
(Study director)


Signature

26-08-99
Date (dd-mm-yy)

B.P.M. Janszen, DVM, PhD
(Head, Department of ILOB)


Signature

26-08-99
Date (dd-mm-yy)

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4. Quality assurance statement

Study Title : Feeding value of Transgenic rice (Event Rice 62) in Poultry-Broilers.
 Report number : V99.034
 Report date : 26/08/1999

The protocol was inspected by the Quality Assurance Unit of TNO Nutrition and Food Research Institute as follows:

Dates of inspection:	Item:	Dates of Report:
08/04/1999	draft protocol	08/04/1999
13/04/1999	protocol	14/04/1999

The experimental phase of this study was inspected by the Quality Assurance Unit of TNO Nutrition and Food Research Institute as follows:

Dates of inspection:	Item:	Dates of Report:
08/04/1999	registration testfeed production	08/04/1999
13/04/1999	division and weighting birds	14/04/1999
20/04/1999	weighing feed, (wing) tagging birds	20/04/1999
18/05/1999	Day 35, weighing of birds	18/05/1999
25/05/1999	weighing and slaughter	25/05/1999

This report was audited by the Quality Assurance Unit of TNO Nutrition and Food Research Institute as follows:

Dates of audit:	Item:	Dates of Report:
28/07/1999 - 02/08/1999	draft report	02/08/1999
27/08/1999	final report	27/08/1999

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I, the undersigned, hereby declare that this report provides an accurate record of the procedures employed and the results obtained in this study; all inspections were reported to the Study Director and the Departmental management on the dates indicated.



Ing. P.A. de Lang
(Quality Assurance Unit)

Date: 27 August 1999

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5. Introduction

5.1 GM rice

GM rice is genetically modified rice which has been modified solely for the purpose of making it resistant to the herbicide glufosinate ammonium. The GM rice in this study was Event Rice 06, with the legal name LLRice 62. In this report, the name GM rice Event 62 will be used for the GM rice.

5.2 Objectives

The aim of the present study was to determine the nutritional equivalence of GM rice Event 62 and non-GM rice in diets of broilers chickens by investigation of :

1. Zootechnical performance
2. Health
3. Slaughter quality

5.3 Study design

This was a nutritional equivalence study. The study was designed to demonstrate the nutritional equivalence of GM rice event 62 and non-GM rice.

5.4 Study parameters (per bird and/or per replicate)

5.4.1 Zootechnical parameters

- individual weight gain/body weight
- feed intake
- feed conversion efficiency (weight gain / feed intake)
- mortality rate

Periods: Day 0-7 / Day 7-14 / Day 14-21 / Day 21-26 / Day 26-35 / Day 35-42;
Weighing broilers and feed on Day 0, 7, 14, 21, 26, 35 and 42 in the morning.

5.4.2 Health

- general health observations: behaviour and physical condition
- clinical inspections
- post mortem examinations

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5.4.3 Slaughter quality

- carcass weight incl. abdominal fat
- breast (Pectoralis) muscle weight incl. bone, excl. skin
- abdominal fat weight

5.5 Study outline

One hundred and twenty, one day old, male broiler chickens were included on Day 0 after a general clinical health inspection by the veterinarian investigator.

The study was carried out with two groups of 60 broiler chickens, subdivided into three replicates of 20 birds. They were housed in an experimental unit at TNO Wageningen. Body weight was determined on Day 0 before inclusion and body weight and feed intake on Day 7, 14, 21, 26, 35 and 42.

Test products/diets were administered from allocation on Day 0 until weighing on Day 42. From Day 0 until Day 26, birds received a starter/grower diet and from Day 26 onwards, they were fed with a finisher diet. Both diets for group A contained 30% non-GM rice, whereas group B was fed diets containing 30% GM rice Event 62.

Twice daily the birds were inspected for general health by an animal technician. Twice weekly the birds were clinically inspected by a veterinarian.

All birds were slaughtered on Day 42. Per bird carcass weight, weight of breast muscle incl. bone and weight of abdominal fat were determined.

A schedule of assessments is given in Annex 1 of the protocol.

5.6 Approval animal experimental committee

Approval to carry out the experiment in this protocol were according to the guidelines by the DEC-TNO, under number 575.

5.7 Conduct of the study

The study was conducted:

1. In compliance with this study protocol
2. In compliance with the OECD principles of Good Laboratory Practice (GLP) 1997. Organization for Economic Co-operation and Development (OECD), Paris. [1]
3. Analysis feed Pre-Mervo: in compliance with 'Sterlab' (Appendix B)
4. Gross energy was determined by the TNO-ILOB laboratory, which is not working in compliance with GLP.

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Deviations from the study protocol and of the applicable SOP's were reported to the study director. The study director and study monitor have evaluated the deviations and decided whether corrective actions were required.

5.8 Quality assurance

The Quality Assurance Unit of TNO Nutrition and Food Research Institute has:

1. Inspected the study protocol, the conduct of the study, including preparation of the diets, and the study report.
2. Reported the findings to the study director and the facility management.
3. Promulgated a quality assurance statement, specifying dates of inspections. and reports to the study director and the management.

5.9 Time schedule of the study

Start in-life phase:	13-04-1999
Termination in-life phase:	25-05-1999
Draft report:	July 1999
Final report:	August 1999

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6. Test system and test product

6.1 Test system/birds

The study was conducted with broiler chickens (crossbred "Cobb") one day old at the start of the experimental period. The broiler chicken served as target species in this study.

In total 140 one-day old broilers (all males) were purchased from Cobroed, Lieveelde, the Netherlands (at 13/04/1999). Ten birds were sent on Day 0 to the Health Service (Deventer) for general pathological check.

At the breeder, before delivery, the birds were vaccinated for Newcastle disease (13/04/1999). All birds were vaccinated for the second time against Newcastle Disease at Day 21 (04/05/1999) using spray / aerosol method.

Two groups of 60 birds were treated in parallel with the test products.

At Day 7, all chickens were identified by a unique animal identification number in both wings. In case an identification number was lost, a new number was attached if possible.

For inclusion the birds met the following criteria:

- Clinically healthy as judged by the veterinarian investigator

Post mortems were conducted on any mortalities to determine whether or not there was any evidence of toxicity.

6.2 Test product

The sponsor supplied sufficient amounts of non-transgenic counterpart rice (240 kg) and GM rice Event 62 (233 kg), which arrived in 9 and 10 drums respectively at 12/03/1999. The TNO registration numbers were TB 603 A through J for the drums with GM rice Event 62 and TB 605 A through I for the drums with the non-transgenic rice.

The GM rice Event 62 had not been 'cleaned' on arrival at TNO, (at 26/03/1999) this was performed at TNO Zeist. The rice was cleaned using a Petkus-Linde cleaning apparatus. This apparatus combines aspiration and sieving to remove impurities. Light impurities like husks and dust are removed by aspiration. Coarse impurities (overs of 4.0 mm sieving screen) and fine impurities or small rice kernels (throughs of 1.25 mm sieving screen) were removed by sieving. The impurities were collected and sent back.

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The test substances (GM rice Event 62 as well as the traditional, non-GM rice) were milled on a hammer mill through a 2.5 mm sieve before mixing through the feed.

The data of the investigational product GM rice Event 62, was summarized as follows:

- Product name : GM rice
- Sample name : Event rice 62 / LLRice 62
- Expiry date : June 2000
- Administration : oral; to group B through the feed
- Dosing : 30% on dietary base; Day 0 - 42.
- Supplier : AgrEvo USA Company
- Sample number : BK 98 B122-50P
- Safety : The product will not be used except for this trial. No special precautions are necessary.

The data of the control non-transgenic counterpart rice was summarized as follows:

- Product name : control, non-GM rice
- Sample name : non-transgenic counterpart
- Expiry date : June 2000
- Administration : oral; to group A through the feed
- Dosing : 30% on dietary base; Day 0 - 42.
- Supplier : AgrEvo USA Company
- Sample number : BK 98B122-49P
- Safety : The product will not be used except for this trial. No special precautions are necessary.

6.3 Dosing/Diets

The test substance was administered at constant concentrations in the feed during the entire study. The birds were fed diets with a practical composition, containing 30% rice. From Day 0 until Day 26, birds received a starter/grower diet and from Day 26 onwards, they were fed a finisher diet.

The study comprised two groups, each consisting of 60 birds, subdivided into three replicates (=pens) of 20 chickens each. The control group (group A) received diets with 30% traditional, non-GM rice. The test group (group B) received diets containing 30% GM rice Event 62.

Group	Colour code	Treatment
A	Red	Control group with non-GM rice
B	Blue	Test group with GM rice Event 62

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The diets contained a coccidiostat (Monensin-Na, 100 mg.kg⁻¹) but no antibiotics. Diets were pelleted (2.5 mm diameter) using steam addition. Details of each batch were included in the animal husbandry file. The origin and composition of the diets were decided upon by the (deputy) study director and are included in annex B03 (Composition of the diets and Analysed and calculated composition of the test products).

Diet preparation was according to the SOP no. DHDV/MEN/201 used in the feedmill of ILOB Department.

During the experiment, the diets were fed ad libitum through a feed hopper.

6.4 Housing and animal care

After allocation, the birds were housed in groups of 20 in floor pens under conventional conditions in experimental room 1K03 at the ILOB-complex. During the study, temperature in the experimental room was kept between 20 and 32 °C, while the relative humidity varied between 35 and 70%. During the first week after arrival, additional heating was provided with a lamp above the pens. Temperature and relative humidity of the experimental room were continuously registered with a thermohygrograph.

Lighting was achieved through artificial lighting. Light were turned on 24 hours per day. Light intensity was gradually decreased during the experiment to prevent cannibalism by turning out part of the artificial lighting.

Light intensity at 'broiler-level' per pen was measured at start of the study and when light intensity was changed.

The pens were provided with at least 2 cm of woodshavings as bedding material.

The birds were also allowed to drink water ad libitum. Regular tap water was provided through drinking nipples attached. A copy of the water analysis, carried out by the water company NUON, is included in Appendix C.

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7. Procedures and methods

7.1 Safety requirements

A risk assessment was provided by the sponsor.

No special precautions were needed when handling the test product. They were treated as normal laboratory chemicals.

Samples collected during the study were handled as potentially infectious. They were handled as described in TNO SOP VOE/VEI/021.

All birds of the test group (group B), the diets, the bedding material and the GM rice Event 62 were burned after slaughter and autopsy.

Approval of the authorities for GMO studies at TNO-ILOB was given December 22nd 1998.

The criteria for acceptable levels of contaminants in food and water and principally the analytical specification established respectively by the USEPA Proposed TSCA Health Effects Test Standards and the WHO and European Council Standards were considered in conjunction with internal policy and other published standards. None of the contaminants expected to be present in the diet or water are known to be capable of interfering with the purpose or conduct of the study at the permitted levels.

7.2 Allocation to study treatment

At Day 0, all 140 birds were weighed individually (without link to identification number) and divided into 7 weight classes of 2 grams (<40g, 40g, 41-42g, 43-44g, 45-46g, 47-48g and 48-51g). An equal number of birds from each weight class was randomly distributed among 6 pens. Each pen contained 20 birds. The remaining birds were euthanised. Total pen weight was registered in the study file.

7.3 Zootechnical measurements

- individual body weight was determined at Day 7, 14, 21, 26, 35 and 42.
- at Day 0, after allocation, total body weight per pen was determined
- birds that died during the experiment were weighed and autopsy was performed (=mortality rate).
- feed intake was determined per pen at each time of weighing and where necessary corrected for the feed consumed by birds that died.

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7.4 Health/animal health care

Twice daily (morning and late afternoon) an animal technician conducted a general health inspection (i.e. the behaviour and the physical condition of the birds were observed). If there were any clinical signs of illness, the veterinarian was consulted.

The veterinarian investigator inspected the birds for clinical health at least twice a week. This was either performed during the normal general health inspection, or separate.

7.5 Slaughter quality

Slaughter Quality All birds were slaughtered on Day 42 at the ILOB Department. The birds were killed by capacititation, exsanguination and then feathers were removed by plucking after soaking with hot water to make it easier.

The following measurements were taken:

- carcass weight (= carcass without head, legs, blood, feathers, organs and intestinal tract and inclusive abdominal fat).
- abdominal fat weight
- breast muscle weight incl. bone , excl. skin
- clinical signs and macroscopic findings were recorded by a pathologist, the abnormalities recorded are shown in Table 1.

Table 1. Recorded abnormalities after slaughter.

code	abnormalities
0	no abnormalities
1	pale liver
2	dilatated heart
3	hydropericard
4	haemorrhagia duodenum / jejunum
5	mucosa gastrum too bubbly
6	haemorrhagia pancreas
7	ascitis

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7.6 Analysis of the test diets

The diets, starter/grower and finisher, of test group A and B were analysed for contents to confirm the composition:

- dry matter
- crude protein
- ash
- crude fat
- Calcium
- Phosphorus

by Pre-Mervo, Protonweg 10, Utrecht, the Netherlands.

Energy of the diets was determined at the ILOB Department.

Samples of all diets were sent to the sponsor for additional analysis. Also samples of all diets are retained at the ILOB Department for a period of 2 years (below -18 °C).

7.7 Labelling of samples

Samples were labelled as follows:

- TNO Nutrition and Food Research
- Study code : 805
- Sample type : starter/grower diet and test product sample number
- Group : A/B
- Sample date : dd-mm-yy
- Sample code : study code/group/sample number

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7.8 Analysis of the data

The individual body weight and slaughter data were analysed statistically using the GLM (general linear models) procedure from SAS (statistical analyses system). The model used was:

$$Y = \mu + group_i + replicate_j + error_{ijk}$$

where: Y : weight
μ : general mean
group : effect of rice type in diet (i=1,2)
replicate : effect of replicate (j=1,2,3)
error : the error for observation k of the ith group and the jth replicate, which is assumed to be independently and normally distributed with a mean value equal to 0 and variance equal to σ^2

For significant effects ($P < 0.05$), expected values per group and replicate were estimated using the LSMEANS step, which corrects for other variables in the model. In all other occasions, the mean values per group were given.

The variables measured per pen (feed intake, feed conversion, mortality) were analysed statistically using a two-sample T-test.

The macroscopic findings were analysed using a paired T-test.

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8. Documentation and retention of records

The documentation of this study consisted of the study protocol, correspondence, report and raw data or true copies of these.

The following documents are retained during 15 years after completion of the final report in the archives of TNO Nutrition and Food Research Institute:

1. Master copies of the approved study protocol and final report
2. Raw data or true copies of these
3. Correspondence
4. All other information related to the study

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9. Deviations of the protocol

The trial was carried out in accordance with the trial protocol, with the exception of the following deviations:

- 1) There was an inconsistency regarding the diets names: the diets fed were a starter/grower diet from Day 0 to 26 and a finisher diet from Day 26 to 42. Not a starter diet for Day 0 to 26 and a grower diet for Day 26 to 42 as mentioned in the protocol.
No influence on the results of the trial.
- 2) The allocation classes were 2 grams instead of 5 grams.
No influence on the results of the trial.
- 3) On 27-4, 28-4, 29-4, 6-5 and 7-5 the General Health Inspection was only performed once (in the morning) instead of twice.
No influence on the results of the trial.
- 4) Body weight and slaughter data were analysed with the GLM procedure from SAS, as this test is more sensitive. The pen data were analysed with a T-test as mentioned in the protocol.
No influence on the results of the trial.

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10. References

- 1) The OECD principles of Good Laboratory Practice 1997. Organization for Economic Co-operation and Development (OECD), Paris. ENV/MC/CHEM (98)17.

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11. Results

11.1 Diets

The results of the diets analyses for dry matter, crude protein, crude fat, ash, calcium, phosphorus and energy contents is shown in Table 2.

Table 2. Analysed contents of the starter/grower and the finisher diets, for both treatment groups (non-GM rice A and GM rice Event 62 B).

	starter/grower A	finisher A	starter/grower B	finisher B
dry matter (g/kg)*	890	891	893	891
crude protein (g/kg)*	212	203	210	204
crude fiber (g/kg)*	81	97	78	99
ash (g/kg)*	63	55	63	55
Calcium (g/kg)*	10.3	8.5	10.2	8.6
Phosphorus (g/kg)*	7.9	6.5	7.9	6.6
GE (MJ/kg)**	17.631	18.228	17.428	17.983

*) Analysed by Pre Mervo, Utrecht, The Netherlands

**) Analysed by TNO-ILOB laboratory (GE = gross energy)

11.2 Health

No abnormalities were found during the general health inspections.

11.3 Mortality

Two birds died during the experimental period, both from pen 2 treatment B. The first one was found dead on Day 3 due to a disorder of the yolk sac and bleeding. The second (bird 7537) was found dead on Day 37 as a result of acitis. The observations were typical in broiler production.

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11.4 Zootechnical parameters

11.4.1 Individual body weight

No significant ($P>0.05$) differences were obtained regarding body weight between the two groups. The results are given in Table 3.

Table 3. Mean body weights per group and per replicate (pen) at certain weighing times; differences were not significant ($P>0.05$).

group	pen	mean body weight (g)						
		Day 0	Day 7	Day 14	Day 21	Day 26	Day 35	Day 42
A	1	45	148	400	825	1234	2078	2503
A	4	45	152	420	876	1276	2085	2534
A	5	45	154	425	887	1285	2114	2538
A	all	45	152	415	863	1265	2092	2525
B	2	45	153	419	866	1265	2087	2557
B	3	45	161	430	887	1290	2112	2458
B	6	45	149	415	848	1239	2054	2537
B	all	45	154	421	867	1265	2084	2516

The mean body weight gain was calculated per period and cumulative (from each weighing day to Day 0). No significant ($P>0.05$) differences were found between the groups A and B. The results are shown in Table 4.

11.4.2 Feed intake

Feed intake of the birds which died during the trial was estimated using their age, their weight and the feed conversion of the pen they originated from. The estimated feed intake was deducted from the cumulative feed intake at the end of the period in which the bird died.

Feed intake was calculated cumulatively and per period per bird per pen. No significant ($P>0.05$) differences were found in the feed intake from group A and B. The results are shown in Table 4 a and b.

11.4.3 Feed conversion efficiency

Feed conversion efficiency was calculated as weight gain per kg feed intake per bird per pen, this was calculated both cumulatively and per period. No significant ($P>0.05$) differences were found between groups A and B. All results are shown in Table 4 a and b.

Table 4 a. Mean feed intake (g/animal/day), feed conversion efficiency (FCE; g feed intake per g weight gain) and body weight gain (g/animal/day) for each period separately and for the cumulative periods; differences were not significant ($P>0.05$); pen 1,4 and 5 contained animals from group A, and pen 2,3 and 6 contained animals from group B.

Number of animals	pen / group	Day 0-7			Day 7-14			Day 14-21			Day 21-26			Day 26-35			Day 35-42		
		feed intake	weight gain	FCE	feed intake	weight gain	FCE	feed intake	weight gain	FCE	feed intake	weight gain	FCE	feed intake	weight gain	FCE	feed intake	weight gain	FCE
20	1	17.8	14.8	0.833	47.1	36.0	0.764	82.3	60.6	0.737	123.7	81.9	0.662	154.8	93.7	0.606	156.4	60.8	0.389
20	4	18.6	15.3	0.823	49.6	38.3	0.773	88.4	65.1	0.736	126.2	80.0	0.634	155.5	89.9	0.578	161.6	64.1	0.397
20	5	19.0	15.7	0.824	49.3	38.7	0.784	89.7	66.0	0.736	125.1	79.6	0.636	156.6	92.1	0.588	159.8	60.6	0.379
	A	18.5	15.3	0.826	48.7	37.6	0.774	86.8	63.9	0.736	125.0	80.5	0.644	155.6	91.9	0.591	159.3	61.8	0.388
18	2	18.6	15.5	0.833	51.5	37.9	0.737	90.3	63.9	0.707	127.7	79.8	0.625	155.9	91.4	0.586	142.5	67.2	0.472
20	3	20.0	16.7	0.834	51.8	38.4	0.741	89.3	65.3	0.732	121.8	80.6	0.662	156.4	91.3	0.584	154.5	49.4	0.320
20	6	17.7	14.8	0.840	49.5	38.0	0.768	86.0	61.9	0.719	119.1	78.3	0.657	156.2	90.5	0.580	163.7	69.0	0.421
	B	18.8	15.7	0.831	50.9	38.1	0.749	88.5	63.7	0.720	122.8	79.5	0.648	156.2	91.1	0.583	153.9	61.7	0.401

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Table 4 b. Mean feed intake (g/animal/day), feed conversion efficiency (FCE; g feed intake per g weight gain) and body weight gain (g/animal/day) for each period separately and for the cumulative periods, differences were not significant ($P>0.05$); pen 1,4 and 5 contained animals from group A, and pen 2,3 and 6 contained animals from group B.

Number of animals	pen / group	Day 0-7			Day 0-14			Day 0-21			Day 0-26			Day 0-35			Day 0-42		
		feed intake	weight gain	FCE	feed intake	weight gain	FCE	feed intake	weight gain	FCE	feed intake	weight gain	FCE	feed intake	weight gain	FCE	feed intake	weight gain	FCE
20	1	17.8	14.8	0.833	32.5	25.4	0.783	49.1	37.2	0.757	63.4	45.8	0.722	86.9	58.1	0.668	98.5	58.5	0.594
20	4	18.6	15.3	0.823	34.1	26.8	0.786	52.2	39.6	0.758	66.4	47.3	0.713	89.3	58.3	0.653	101.4	59.3	0.585
20	5	19.0	15.7	0.824	34.2	27.2	0.795	52.7	40.1	0.761	66.6	47.7	0.716	89.8	59.1	0.659	101.4	59.4	0.585
	A	18.5	15.3	0.826	33.6	26.5	0.788	51.3	38.9	0.759	65.5	46.9	0.717	88.7	58.5	0.660	100.4	59.1	0.588
18	2	18.6	15.5	0.833	35.0	26.7	0.763	53.5	39.1	0.730	67.7	46.9	0.693	90.4	58.3	0.645	103.3	59.8	0.579
20	3	20.0	16.7	0.834	35.9	27.5	0.767	53.7	40.1	0.747	66.8	47.9	0.717	89.8	59.1	0.657	100.6	57.5	0.571
20	6	17.7	14.8	0.840	33.6	26.4	0.787	51.0	38.2	0.749	64.1	45.9	0.716	87.8	57.4	0.654	100.5	59.3	0.591
	B	18.8	15.7	0.831	34.8	26.9	0.771	52.7	39.1	0.742	66.2	46.9	0.709	89.3	58.3	0.652	101.4	58.8	0.580

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11.5 Slaughter quality

11.5.1 Carcass weight

Carcass weight of the slaughtered birds was determined and the percentage was calculated using the Day 42 body weight (Appendix D). No significant ($P>0.05$) differences were found between group A and B, in either the absolute carcass weight and the percentage, nor was a difference between the pens present. The results are shown in Table 5.

11.5.2 Breast muscle

The breast muscle (including bone, excluding skin) was removed from the carcass and weighed, the percentage was calculated using the Day 42 body weight (Appendix D). No significant ($P>0.05$) differences were found group A and B, in either the absolute weight of the breast muscle or the percentage. The results are shown in Table 5.

11.5.3 Abdominal fat

Abdominal fat was removed from the carcass and weighed. The percentage was calculated using the Day 42 body weight (Appendix D). No significant ($P>0.05$) differences were found between the pens or group A and B, for either the absolute weight or the percentage. The results are shown in Table 5.

11.5.4 Post mortem macroscopic findings

Macroscopic abnormalities found after slaughter were recorded. (In total 3 different abnormalities were found (Table 1), either separately or in combination. The results are shown in Table 6. No abnormalities were observed in half of the birds. For both groups (A and B) a pale liver was the most prevalent abnormality. The *post mortem* macroscopic findings were typical observations in broiler production and are not due to the diets. No significant differences ($P>0.05$) were found between group A and B.

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Table 5. Mean weight of the carcass, the breast and the abdominal fat, and the percentage of the carcass, the breast and the abdominal fat of the Day 42 weight (differences were not significant ($P>0.05$)); pen 1,4 and 5 contained animals from group A, and pen 2,3 and 6 contained animals from group B.

number of animals	pen / group	carcass		breast		abdominal fat	
		(g)	(%)	(g)	(%)	(g)	(%)
20	1	1758.4	70.2%	492.7	19.7%	40.4	1.6%
20	4	1791.8	70.7%	507.6	20.0%	46.3	1.8%
20	5	1808.8	71.2%	503.3	19.8%	51.3	2.0%
	A	1786.3	70.7%	501.2	19.8%	46.0	1.8%
18	2	1798.9	70.3%	501.2	19.6%	48.2	1.9%
20	3	1749.2	71.2%	493.7	20.1%	47.7	1.9%
20	6	1800.6	71.0%	504.2	19.8%	52.5	2.1%
	B	1782.3	70.8%	499.6	19.8%	49.5	2.0%

Table 6. Percentage and number of birds with a specific abnormality (no significant differences ($P>0.05$)), 59 and 58 birds for group A and B respectively. Codes are given in Table 1, paragraph 7.5.

code	all	group A			group B		
	%	%	n	n (single)	%	n	n (single)
0	54.7	54.2	32	32	55.2	32	32
1	37.6	40.7	24	19	34.5	20	18
2	4.3	3.4	2	1	5.2	3	3
3	9.4	10.2	6	2	8.6	5	3

n = number of times observed in a bird

n (single) = number of times as separate observation

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12. Conclusion

No significant ($P > 0.05$) differences were found between the birds receiving the diet containing normal rice or GM rice Event 62, LLRice 62. During the entire period, feed intake, weight gain and feed conversion were similar in all pens. Further, the carcass quality of all birds was similar.

Therefore it can be concluded that GM rice Event 62 (LLRice 62) was nutritionally equivalent to non-GM rice.

Appendix A Protocol

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TNO protocol
DHDV/PRT/805
PV I 99.014

Feeding value of Transgenic rice (Event Rice 62) in Poultry-Broilers.

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TNO project number:
010.20505
Study number
805

Status:
FINAL
Previous versions:
none
Number of pages:
21
Number of annexes:
3
Number of forms:
none

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Netherlands Organization for Applied Scientific Research (TNO)

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Approval

Sponsor

AgrEvo UK Limited

Dr C. Reynolds
(Study monitor)


Signature

7/4/99
Date (dd-mm-yy)

Testing facilities

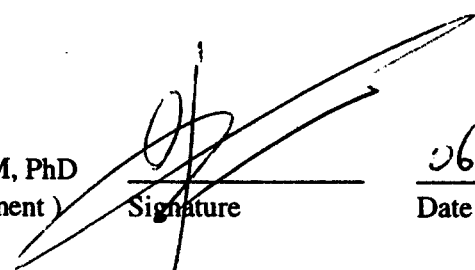
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ILOB Department

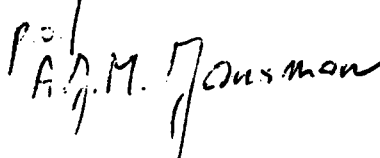
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(Study Director)


Signature

06 04 99
Date (dd-mm-yy)

B.P.M. Janszen, DVM, PhD
(Head, ILOB Department)


Signature



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Date (dd-mm-yy)

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List of abbreviations

b.w	Body weight
DHDV	Human and Animal Nutrition Division
GM	Genetically modified
GMO	Genetically modified organisms
I&R	Identification and Registration number
SOP	Standard operating procedure

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1 Summary

To investigate the nutritional equivalence of the test products GM (Genetically modified) rice (Event Rice 62) and its non transgenic counterpart (non-GM) rice in broiler chickens, the zootechnical performance, general health and slaughter quality will be determined in two groups of 60 male broiler chickens. Each group will be subdivided into three replicates (=pens) of 20 broilers each.

The control group, group A, will receive diets with 30% non-GM rice. The test group, group B, will receive diets containing 30% GM rice.

The parameters of interest during the in-life phase will be:

- the behaviour and the physical condition of the birds will be observed twice daily by general health inspection.
- individual body weight will be determined at Day 0, 7, 14, 21, 26, 35 and 42.
- at Day 0, after allocation, total body weight per pen will be determined within two hours after arrival.
- mortality rate
- feed intake will be determined per pen at each time of weighing.

From body weight and feed intake, feed conversion efficiency will be calculated.

The birds will be slaughtered at Day 42, the parameters of interest will be:

- carcass weight incl. abdominal fat
- breast muscle weight incl. bone excl. skin
- abdominal fat weight

Clinical signs and macroscopic findings will be recorded by a pathologist.

To determine the nutritional equivalence of the two test products the data of two test groups will be analysed statistically using a T-test.

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2 Responsible personnel and testing facilities

2.1 Sponsor

Sponsor:
AgrEvo UK Limited
Chesterford Park
Saffron Walden
Essex CB10 1XL, England, UK
Phone: + 44 (0)1799 530123
Fax: + 44 (0)1799 573546

Study monitor :
Dr. C. Reynolds
Phone: +44 (0) 1799 573527
Fax: + 44 (0) 1799 573546
email: caroline.reynolds@agrevo.com

2.2 Testing facilities

The study will be performed at:

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Human and Animal Nutrition Division (DHDV)
Department of ILOB
Haarweg 8
P.O. Box 15
6700 AA Wageningen
Phone:+31 317 499 440
Fax: +31 317 499 463
e-mail: schat@voeding.tno.nl

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2.3 Responsibilities

The sponsor will be responsible for the supply of sufficient test product.

Study director	:B. Schat DVM B.Sc (1) Phone: + 31 317 499 482 Email:schat@voeding.tno.nl
Deputy study director	:G.M. Beelen B.Sc (1) Phone: + 31 317 499 410 Email:beelen@voeding.tno.nl
Responsible for animal care	: K. Deuring, D. van Kleef, J. van Harn, J. de Jong, A. Hoek. (1)
Responsible for diet preparation/ feedmill	:P.C. Roeleveld, C. Siebers
Responsible for slaughtering	: J. van Harn (1)
Responsible for veterinarian investigation	: B. Schat, B.P.M. Janszen (1), H.A.M. van Elst DVM, E.J. Kiemeneij DVM (3)
Responsible for statistics	: J. Wiebenga B.Sc (1)
Responsible for pathology	: M.V.W wijnands DVM, M.H.M. Bos- Kuijpers, DVM PhD (2)
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(4) Pre-Mervo
Protonweg 10, Utrecht

2.4 Subcontracting

The analyses of the diets (dry matter, crude protein, ash, crude fat, Calcium and Phosphorus) will be subcontracted at/by Pre-Mervo, Protonweg 10, Utrecht, the Netherlands.

3 Introduction

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3.1 GM rice

GM rice is genetically modified rice which have been modified solely for the purpose of making it resistant to the herbicide, glufosinate ammonium.

3.2 Objectives

The aim of the present study is to determine the nutritional equivalence of GM rice and non-GM rice in diets of broilers chickens by investigation of :

1. Zootechnical performance (see 3.4.1)
2. Health
3. Slaughter quality

3.3 Study design

This is a nutritional equivalence study. The study is designed to demonstrate the nutritional equivalence of GM-rice event 62 and non-GM rice.

3.4 Study parameters (per animal and/or per replicate)

3.4.1 Zootechnical parameters

- individual weight gain/body weight
- feed intake
- feed conversion efficiency (weight gain / feed intake)
- mortality rate

Periods: Day 0-7/Day 7 - 14/Day 14-21/ Day 21-26/Day 26-35/ Day 35-42;
Weighing broilers and feed on Day 0, 7, 14, 21, 26, 35 and 42 in the morning.

3.4.2 Health

- general health observations: behaviour and physical condition
- clinical inspections
- post mortem examinations

3.4.3 Slaughter quality

- carcass weight incl. abdominal fat
- breast (Pectoralis) muscle weight incl. bone, excl. skin
- abdominal fat weight

3.5 Study outline

One hundred and twenty, one day old, male broiler chickens will be included on Day 0 after a general clinical health inspection by the veterinarian investigator. The study will be carried out with two groups of 60 broiler chickens, subdivided

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into three replicates of 20 animals. They will be housed in an experimental unit at TNO Wageningen. Body weight will be determined on Day 0 before inclusion and body weight and feed intake on Day 7, 14, 21, 26, 35 and 42.

Test products/diets will be administered from allocation on Day 0 until weighing on Day 42.

From Day 0 until Day 26, birds will receive a starter diet and from Day 26 onwards, they will be fed with a grower diet.

Both diets for group A will contain 30% non-GM rice, whereas group B will be fed diets containing 30% GM rice(Event rice 62).

Twice daily the animals will be inspected for general health by an animal technician. Twice weekly the animals will be clinically inspected by a veterinarian.

All animals will be slaughtered on Day 42. Per animal carcass weight, breast muscle incl. bone, abdominal fat and liver weight will be determined.

A schedule of assessments is given in Annex 1.

3.6 Approval Animal Experimental Committee

Approval to carry out the experiment in this protocol will be according to the guidelines by the DEC-TNO.

3.7 Conduct of the study

The study will be conducted:

1. In compliance with this study protocol
2. In compliance with the OECD principles of Good Laboratory Practice (GLP) 1997. Organization for Economic Co-operation and Development (OECD), Paris. [1]
3. Analysis feed Pre-Mervo: in compliance with 'Sterlab' (explanation given in final report)

Amendments to the authorised protocol will be documented, the reasons for the changes or revisions being stated, signed and dated by the study director, principal investigator (if involved) and the management, and then sent to the sponsor for approval. Amendments will be retained with the original protocol.

Deviations from the study protocol and of the applicable SOP's will be reported to the study director. The study director and study monitor will evaluate the deviations and decide whether corrective actions will be required.

3.8 Quality assurance

The Quality Assurance Unit of TNO Nutrition and Food Research Institute will:

1. Inspect the study protocol, the conduct of the study, including preparation of

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the diets, and the study report.

2. Report the findings to the study director and the facility management.
3. Promulgate a quality assurance statement, specifying dates of inspections, and reports to the study director and the management.

3.9 Time schedule of the study

Start in-life phase:	Week 15 1999, April 13th
Termination in-life phase:	Week 20 1999
Draft report:	6 weeks after termination in-life phase
Final report:	10 weeks after term. in life-phase

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4 Test system and test product

4.1 Test system/birds

The study will be conducted with broiler chickens (crossbred "Cobb") one day old at the start of the experimental period. The broiler chicken serves as target species in this study.

In total 140 one-day old broilers (all males) will be purchased from Cobroed, Lievelede, the Netherlands. Ten birds will be sent on Day 0 to the Health Service for general pathological check.

At the breeder, the birds will be vaccinated for Newcastle disease. All birds will be vaccinated for the second time against Newcastle Disease at Day 13 - 15 using spray method (will be specified in final report).

Two groups of 60 birds will be treated in parallel with the test products.

At Day 7, all chickens will be identified by a unique animal identification number in both wings. In case an identification number is lost, a new number will be attached if possible.

For inclusion the birds should meet the following criteria:

- Clinically healthy as judged by the veterinarian investigator

Post mortems will be conducted on any mortalities to determine whether or not there is any evidence of toxicity.

4.2 Test product

The sponsor will supply at least 80 - 100 kg of non-transgenic counterpart rice and 80 - 100 kg GM rice, Event rice 62.

The test substances (GM rice as well as the traditional, non-GM rice) will be milled on a hammer mill through a 2.5 mm sieve before mixing through the feed.

The data of the investigational product GM rice, transgenic event 62, can be summarized as follows:

- Product name :GM rice
- Sample name :Event rice 62
- Claimed composition :To be given in final report
- Administration :oral; to group B through the feed
- Dosing :30% on dietary base; Day 0 - 42.
- Supplier :AgrEvo USA Company
- Sample number :BK 98 B122-50P

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- Safety :The product will not be used except for this trial

The data of the control non-transgenic counterpart rice can be summarized as follows:

- Product name :control, non-GM rice
- Sample name :non-trangenic counterpart
- Claimed composition :To be given in final report
- Administration :oral; to group A through the feed
- Dosing :30% on dietary base; Day 0 - 42.
- Supplier :AgrEvo USA Company
- Sample number :BK 98B122-49P
- Safety : The product will not be used except for this trial. No special precautions necessary.

4.3 Dosing/Diets

The test substance will be administered at constant concentrations in the feed during the entire study. The control group will receive non-GM rice in the diets.

During the entire experiment, the birds will be fed diets with a practical composition, containing 30% rice. From Day 0 until Day 26, birds will receive a starter diet and from Day 26 onwards, they will be fed a grower diet.

The study will comprise two groups, each consisting of 60 birds, subdivided into three replicates (=pens) of 20 chickens each. The control group (group A) will receive diets with 30% traditional, non-GM rice. The test group (group B) will receive diets containing 30% GM rice.

Group	Colour code	Treatment
A	Red	Control group with non-GM rice
B	Blue	Test group with GM rice (event 62)

The diets will contain a coccidiostat (Monensin-Na, 100 mg.kg⁻¹) but no antibiotic. Diets will be pelleted (2.5 mm diameter) using steam addition. Details of each batch will be included in the animal husbandry file. The origin and composition of the diets are decided upon by the (deputy) study director and are included in annex B03 (Composition of the diets and Analysed and calculated composition of the test products).

Diet preparation will be according to the SOP no. DHDV/MEN/201 used in the feedmill of ILOB Department.

During the experiment, the diets will be fed ad libitum through a feed hopper.

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4.4 Housing and animal care

After allocation, the birds will be housed in groups of 20 in floor pens under conventional conditions in experimental room 1K03 at the ILOB-complex. During the study, temperature in the experimental room will be kept between 20 and 32 °C. During the first week after arrival, additional heating will be provided with a lamp above the pens. Temperature and relative humidity of the experimental room will be continuously registered with a thermohydrograph.

Lighting will be achieved through artificial lighting. Light will be turned on 24 hours per day. Light intensity will be gradually decreased during the experiment to prevent cannibalism by turning out part of the artificial lighting. Light intensity on 'broiler-level' per pen will be measured at start of the study and when light intensity will be changed.

The pens are provided with at least 2 cm of woodshavings as bedding material.

The birds will also be allowed to drink water ad libitum. Regular tap water will be provided through drinking nipples attached to a container. A copy of the water analysis, carried out by the water company NUON, will be included in the study file.

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5 Procedures and methods

5.1 Safety requirements

A risk assessment will be provided by the sponsor.

No special precautions will be needed when handling the test product. They may be treated as normal laboratory chemicals.

Samples collected during the study will be handled as potentially infectious. They will be handled as described in TNO SOP VOE/VEI/021.

All birds of the test group (group B) and the GM rice will be burned after slaughter and autopsy.

Approval of the authorities for GMO studies at TNO-ILOB was given December 22 1998.

The criteria for acceptable levels of contaminants in food and water and principally the analytical specification established respectively by the USEPA Proposed TSCA Health Effects Test Standards and the WHO and European Council Standards considered in conjunction with internal policy and other published standards. None of the contaminants expected to be present in the diet or water are known to be capable of interfering with the purpose or conduct of the study at the permitted levels .

5.2 Allocation to study treatment

5.2.1 Group

At Day 0, all 140 birds will be weighed individually (without link to identification number) and divided into weight classes of 2 grams. After weighing all the birds, per weight class an equal number of birds will be randomly distributed among the six pens. Each pen will contain 20 birds. The remaining birds will be euthanised. Total pen weight will be registered.

5.3 Zootechnical measurements

- individual body weight will be determined at Day 0, 7, 14, 21, 26, 35 and 42.
- at Day 0, after allocation, total body weight per pen will be determined
- birds that die during the experiment will be weighed and autopsy will be performed (=mortality rate).
- feed intake will be determined per pen at each time of weighing.

C 0 0 5 1 4 8

5.4 Health/Animal health care

Twice a day (morning and late afternoon) an animal technician will conduct a general health inspection (= the behaviour and the physical condition of the birds will be observed). If there are any clinical signs of illness, the veterinarian will be consulted.

The veterinarian investigator will inspect the birds for clinical health at least twice a week.

5.4.1 Slaughter Quality

All birds will be slaughtered on Day 42 at the ILOB Department.

The birds will be killed by cervical dislocation, exsanguination and then feathers will be removed by plucking after sprinkling with hot water to make it easier.

The following measurements will be taken:

- carcass weight (= carcass without head, legs, blood, feathers, organs and intestinal tract and inclusive abdominal fat.
- abdominal fat weight
- breast muscle weight incl. bone, excl. skin
- clinical signs and macroscopic findings will be recorded by a pathologist.

5.5 Analysis of the test diets

The diets, starter and grower, of test group A and B will be analysed for contents to confirm the composition:

- dry matter
- crude protein
- ash
- crude fat
- Calcium and
- Phosphorus

by Pre-Mervo, Pretonweg 10, Utrecht, the Netherlands.

Energy of the diets will be determined at the ILOB Department.

Samples of all diets will be sent to the sponsor for additional analysis. Also samples of all diets will be retained at the ILOB Department for a period of 2 years (below -18 °C).

5.6 Labelling of samples

Samples will be labelled as follows:

- TNO Nutrition and Food Research

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- Study code: 805
- Sample type starter/grower diet and testproduct sample number
- Group A/B
- Sample date dd-mm-yy
- Sample code 'study code/group/sample number

5.7 Analysis of the data

The data will be analysed statistically using a T-Test.

6 Documentation and retention of records

The documentation of this study will consist of the study protocol, correspondence, report and raw data or true copies of these.

The following documents will be retained during 15 years after completion of the final report in the archives of TNO Nutrition and Food Research Institute:

1. Master copies of the approved study protocol and final report
2. Raw data or true copies of these
3. Correspondence
4. All other information related to the study

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7 Reporting and publication

A final report will be prepared including details about

1. The objective of the study
2. Characterization of the test materials
3. Testing facility and responsible personnel
4. Dates on which the study was initiated and completed
5. Methods, observations, measurements and statistical methods
6. Evaluation of the results
7. Where appropriate, discussion and conclusions
8. Location of the raw data and the final report

The final report will contain a quality assurance statement by the Quality Assurance Unit of the TNO Nutrition and Food Research Institute and a statement on GLP compliance by the study director.

Publication rights of the data remain with the sponsor. Any data referring to TNO will be published or made accessible to the public only after mutual agreement between the sponsor and TNO. TNO will not unreasonably withhold publication.

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8 References

1. The OECD principles of Good Laboratory Practice 1997.
Organization for Economic Co-operation and Development (OECD), Paris.
ENV/MC/CHEM (98)17.

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9 List of addenda

9.1 Annexes

B01. Schedule of assessments

B02. Distribution list

B03. Analysed (*) and calculated composition of the testproducts in g/kg.

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Schedule of assessments

Day	Clock time hh:mm	Action	Registration form
00	16:00	Arrival of the birds Clinical health inspection Weighing birds(indiv.) Allocation to group/replicate Weighing birds(per pen) Start Starter Diet General health inspection	DHDV/PRT/805 F13 DHDV/PRT/805 F04 DHDV/PRT/805 F05 DHDV/PRT/805 F03
01	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
02	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
03	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
04	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
05	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
06	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
07	09:00 09:30 16:00	General health inspection Weighing birds Identification of the birds Weighing feed General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 04 DHDV/PRT/805 F 06 DHDV/PRT/805 F 03
08	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
09	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
10	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
11	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03

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Schedule of assessments

Day	Clock time hh:mm	Action	Registration form
12	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
13	09:00 16:00	General health inspection General health inspection	DHHV/PRT/805 F 03 DHDV/PRT/805 F 03
14	09:00 09.30 16.00	General health inspection Weighing birds Weighing feed General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 04 DHDV/PRT/805 F 06 DHDV/PRT/805 F 03
15	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
16	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
17	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
18	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
19	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
20	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
21	09:00 09:30 16:00	General health inspection Weighing birds Weighing feed General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 04 DHDV/PRT/805 F 06 DHDV/PRT/805 F 03
22	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
23	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03

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Schedule of assessments

Day	Clock time hh:mm	Action	Registration form
24	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
25	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
26	09:00 09:30 16:00	General health inspection Weighing birds Weighing feed, remove starter diet Start Grower diet General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 04 DHDV/PRT/805 F 06 DHDV/PRT/805 F 03
27	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
28	09:00 16:00	General health inspection Weighing birds Weighing feed General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 04 DHDV/PRT/805 F 06 DHDV/PRT/805 F 03
29	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
30	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
31	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
32	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
33	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
34	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03

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Schedule of assessments

Day	Clock time hh:mm	Action	Registration form
35	09:00 09:30 16:00	General health inspection Weighing birds Weighing feed General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 04 DHDV/PRT/805 F 06 DHDV/PRT/805 F 03
36	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
37	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
38	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
39	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
40	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
41	09:00 16:00	General health inspection General health inspection	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03
42	09:00 09:30	General health inspection Weighing birds Weighing feed Slaughtering birds END of in LIFE-PHASE	DHDV/PRT/805 F 03 DHDV/PRT/805 F 03 DHDV/PRT/805 F 06 DHDV/PRT/805 F 07/08

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Distribution list

TNO:

- B. Schat
- B.P.M. Janszen
- G.M. Beelen
- K. Deuring
- D. van Kleef
- J. van Harn
- J. de Jong (Animal Registration)
- P.C. Roeleveld / C.Siebers
- M.V.W. Wijnands
- Quality Assurance Unit
- Log book
- W. Bogaerts (Biological safety)

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Analysed (*) and calculated composition of the test products in g/kg for study 805

	BK98B122-49P	BK98B122-50P
Dry matter*	882	885
Ash*	42	39
Crude protein*	72	78
Crude fat*	22	22
Crude fibre*	88	94
Other carbohydrates	658	652
Met. Energy broilers (kcal/kg) [#]	2642	2638
Met. Energy poultry (kcal/kg) [#]	2740	2739
Calcium	0.4	0.4
Phosphorus, total	2.6	2.6
Phosphorus, dig.	0.4	0.4
Sodium	0.3	0.3
Potassium	3.4	3.4
Chloride	1.0	1.0
Lysine, total	3.0	3.3
Lysine, dig.	2.0	2.2
Methionine, total	1.5	1.6
Methionine, dig.	1.3	1.4
Meth. + Cyst., total	3.1	3.4
Meth. + Cyst., dig.	2.6	2.8
Threonine, total	2.7	2.9
Threonine, dig.	1.9	2.1
Tryptophan, total	0.8	0.9
Tryptophan, dig.	0.7	0.7

* based on values determined in broilers. These specific values are used in the Netherlands

[#] based on values determined in adult roosters. These values are commonly used outside the Netherlands

Both formulas use contents of crude protein, crude fat and carbohydrates -/- crude fibre and their digestibility coefficients to estimate the metabolizable energy content.

All calculated values are based on those of rice mentioned in the CVB table of 1998 (commodity board for feedstuffs, the Netherlands). Amino acid contents are corrected for crude protein content.

DHDV/PRT/805 B03
6 April 1999**Composition of the diets (in g/kg) of study 805**

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	Start/Grower (0-26 d)		Finisher (26-42 d)	
	A	B	A	B
Rice BK98B122-49P	300.00	-	300.00	-
Rice BK98B122-50P	-	300.00	-	300.00
Corn	274.80	280.80	282.40	288.30
Soya beans, heat treated	50.00	50.00	50.00	50.00
Soyabeanmeal	170.00	165.00	147.50	142.50
Rapeseedmeal	50.00	50.00	50.00	50.00
Fishmeal	15.00	15.00	15.00	15.00
Feathermeal (hydr.)	15.00	15.00	15.00	15.00
Meat meal tankage	50.00	50.00	50.00	50.00
Animal fat	10.00	10.00	10.00	10.00
Soya oil	29.00	28.00	51.00	50.00
Premix (corn)*	10.00	10.00	10.00	10.00
Limestone	10.30	10.30	8.20	8.20
Monocalciumphosphate	9.50	9.50	4.30	4.30
Salt (NaCl)	2.00	2.00	2.00	2.00
L-lysine HCl	2.10	2.20	2.40	2.50
DL-methionine	2.10	2.00	1.90	1.90
L-tryptophan	0.20	0.20	0.30	0.30
Total	1,000.00	1,000.00	1,000.00	1,000.00
Calculated contents (g/kg)				
Crude protein	216	216	207	207
Ash	66	65	58	57
Crude fat	79	78	101	100
Crude fibre	48	50	48	49
Calcium	9.5	9.5	7.8	7.8
Phosphorus, total	7.2	7.2	5.9	5.9
Phosphorus, dig.	4.0	4.0	2.9	2.9
Sodium	1.5	1.5	1.5	1.5
Potassium	7.9	7.8	7.4	7.3
Chloride	2.7	2.8	2.8	2.8
Linoleic acid	30	30	42	15
Met. Energy broilers (kcal/kg)	2,800	2,800	2,970	2,970
Met. Energy poultry (kcal/kg)	3,000	3,000	3,175	3,175
Lysine, digestible	11.0	11.0	10.7	10.7
Meth. + Cyst., digestible	8.1	8.1	7.7	7.7
Threonine, digestible	6.7	6.7	6.4	6.3
Tryptophan, digestible	2.2	2.2	2.2	2.2

All diets will be supplemented with 100 mg/kg Monensin-Na

* Premix supplies per 1 kg of diet : 10,000 IU vit. A, 2,000 IU vitamin D₃, 15 mg vitamin E, 5 mg riboflavin, 40 mg niacin amide, 12 mg d-pantothenic acid, 500 mg choline chloride, 15 µg vitamin B₁₂, 5 mg vitamin K, 0.75 mg folic acid, 0.1 mg biotin, 1 mg CoSO₄·7H₂O, 0.15 mg Se (Selplex50), 300 mg FeSO₄·7H₂O, 60 mg CuSO₄·5H₂O, 100 mg MnO₂, 150 mg ZnSO₄·H₂O, 100 mg Ethoxyquin.

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Appendix B

Appendix B

Accreditation certificate Pre-Mervo

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ACCREDITATION CERTIFICATE

The Dutch Council for Accreditation operating as accreditor for test laboratories under the name STERLAB, hereby declares that

Pre-Mervo Kwaliteitsdienst Utrecht

complies with the accreditation criteria for laboratories as described in the STERLAB Criteria which contain all of the criteria from EN 45001 and ISO/IEC guide 25 and the relevant criteria from ISO 9001/9002. The accreditation covers the quality system of the laboratory as well as the specified activities described in the schedule which is provided with the accreditation certificate bearing the accreditation number.

The accreditation will remain valid until further notice provided that the laboratory continues to meet the criteria as laid down by the Dutch Council for Accreditation.

This certificate with accreditation number:

L 166

is granted on

3 October 1994

On behalf of the Dutch Council for Accreditation

The General Manager



The Manager Operations



95567 3/5/95
700 B
Aankman



Appendix C

Water quality analyses

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WATER		Norm	WettIn	Wageningen
Rein water 1998				
Wett. ond. freq. 1				
Aluminium	µg/l	200	30	<5
Arseen	µg/l	50,0		3,6
Barium	µg/l	500	100	<5
Cadmium	µg/l	5,0		<0,2
Chroom	µg/l	50,0		<1
Kwik	µg/l	1,00		<0,05
Lood	µg/l	50		<6
Nikkel	µg/l	50		<6
Seleen	µg/l	10		<2
Zink	µg/l	100		<6
Cyanide	µg/l			<6
Fluoride	mg/l			0,05
Wett. ond. freq. 4				
Kleur	mg Pt/l	20	10	3
KMnO ₄ -verbr.	mg/l	21,7	8,0	<1
Chloride	mg/l	150	150	7
Nitraat	mg/l	50	25	<1
Sulfaat	mg/l	150	100	7
Waterstofcarbonaat	mg/l		>120	94
Kooldioxyde	mg/l			2
Carbonaat	mg/l			<1
Fosfaat	mg/l	8,10	<0,5	0,08
Siliciumdioxyde	mg/l			12
Calcium	mg/l	150	100	29
Magnesium	mg/l	50,0	30,0	2,2
Natrium	mg/l	120		5,5
Kalium	mg/l	12,0		0,5
Opg. org. koolstof	mg/l			0,3
Cu opl. verm.	mg/l	3,0	2,0	1,1
Wett. ond. freq. 13				
Geleiding bij 20 °C	mS/m	125	80	17
Nitriet	mg/l	0,10	0,05	<0,02
Ammonium	mg/l	0,20	0,05	<0,05
IJzer	mg/l	0,20	0,05	0,02
Mangaan	mg/l	0,05	0,02	<0,01
Wett. ond. freq. 52				
Troebetheid	FTE	3	0,4	0,2
Temperatuur	°C	25,0	15,0	11,0
pH		7-9,5	8-8,3	8,02
Zuurstof	mg/l	>2	>4	6,3
Kol. getal 22°C		<100		2
Berekende waarden				
Totale hardheid	mmol/l			0,80
Wat. carb. hardheid	mmol/l			0,77
ΣN+S	meq/l			0,15
pH-evenwicht				8,22
Saturatie-index (S.I.)			-0,2<SI<0,3	-0,20
Asvullend onderzoek				
Trichlooretheen	µg/l	1,00		-
Trichloormethaan	µg/l	1,00		-
Tetrachlooretheen	µg/l	1,00		-
1,2 dichlooretheen-cis	µg/l	1,00		-
N,P bestr. middelen	µg/l	0,10		-
Bentazon	µg/l	0,10		-

Appendix D

Individual data

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Body weight (BW), carcass weight, weight of abdominal fat and of the breast muscle are given in grams.

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Appendix D

animal	pen	treatment	BW 7	BW 14	BW 21	BW 26	BW 35	BW 42	carcass	abdominal fat	breast
7309	1	1	152	365	743	1167	1941	2523	1821	32	469
7319	1	1	159	421	884	1294	2095	2468	1778	23	520
7340	1	1	145	332	632	1030	1880	2390	1629	29	449
7345	1	1	170	495	1008	1438	2362	2887	2023	57	580
7359	1	1	144	380	817	1196	1949	2241	1595	30	454
7362	1	1	137	358	728	1105	1935	2528	1753	38	478
7364	1	1	97	178	395	733	1567	2210	1465	47	402
7365	1	1	152	424	872	1292	2197	2628	1857	47	531
7366	1	1	160	419	883	1318	2192	2661	1879	56	520
7368	1	1	137	425	874	1283	2073	2441	1772	40	526
7369	1	1	125	365	746	1168	2074	2594	1812	36	507
7375	1	1	197	509	990	1426	2283	2748	1955	56	534
7380	1	1	116	298	640	1002	1720	2154	1482	27	443
7384	1	1	142	399	819	1176	1947	2248	1542	26	403
7385	1	1	163	455	922	1338	2215	2593	1754	50	491
7386	1	1	178	492	959	1368	2276	2374	1693	35	460
7391	1	1	151	413	828	1207	2045	2439	1717	26	462
7394	1	1	126	336	743	1192	2172	2671	1879	56	495
7397	1	1	179	505	1053	1516	2308	2579	1827	65	538
7398	1	1	138	435	958	1433	2321	2688	1935	32	592
		average	148.4	400.2	824.7	1234.1	2077.6	2503.3	1758.4	40.4	492.7
		stdev	23.30	79.42	154.71	180.94	209.20	193.80	153.82	12.82	51.53
7602	4	1	172	504	1045	1482	2326	2787	1970	40	555
7603	4	1	145	392	858	1249	2105	2647	1823	50	522
7612	4	1	185	445	884	1294	2111	2719	1995	51	590
7616	4	1	136	421	880	1323	2146	2762	1969	61	555
7621	4	1	146	431	832	1212	1922	2244	1631	32	500
7625	4	1	162	445	900	1316	2049	2428	1712	36	483
7627	4	1	146	395	752	1095	1884	2380	1683	50	457
7632	4	1	158	414	938	1358	2176	2536	1819	52	501
7643	4	1	126	338	762	1124	1932	2438	1697	43	471
7644	4	1	155	426	912	1313	2170	2358	1670	36	482
7647	4	1	159	441	940	1340	2092	2547	1852	27	517
7648	4	1	164	459	956	1328	2120	2513	1777	50	533
7650	4	1	128	362	780	1158	1871	2392	1681	53	454
7666	4	1	183	500	978	1403	2180	2413	1662	44	445
7672	4	1	149	408	834	1215	1960	2238	1588	38	429
7673	4	1	147	412	897	1314	2270	2890	1995	46	596
7677	4	1	137	370	773	1189	2060	2637	1897	66	579
7681	4	1	141	372	814	1222	2102	2610	1850	44	553
7693	4	1	144	396	803	1193	2032	2525	1753	52	452
7696	4	1	162	474	980	1388	2194	2611	1811	55	477
		average	152.3	420.3	875.9	1275.8	2085.1	2533.8	1791.8	46.3	507.6
		stdev	16.16	44.05	82.05	99.28	123.89	176.87	126.94	9.66	51.02

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Appendix D

animal	pen	treatment	BW 7	BW 14	BW 21	BW 26	BW 35	BW 42	carcass	abdominal fat	breast
7709	5	1	172	463	1008	1411	2096	2345	1705	31	497
7713	5	1	155	460	984	1472	2349	2848	2132	65	654
7715	5	1	142	390	796	1184	1946	2500	1778	27	511
7721	5	1	148	419	887	1314	2220	2541	1817	45	517
7722	5	1	151	395	874	1278	2085	2315	1635	36	407
7726	5	1	144	428	848	1232	1926	2349	1633	39	451
7727	5	1	157	437	854	1250	2136	2659	1914	33	600
7728	5	1	136	398	869	1273	2100	2387	1669	49	429
7736	5	1	134	388	864	1281	2145	2603	1848	41	505
7744	5	1	172	423	893	1273	2098	2518	1770	62	463
7749	5	1	149	382	875	1309	2210	2638	1919	38	574
7751	5	1	146	372	779	1135	1972	2525	1800	42	505
7753	5	1	178	488	890	1277	2079	2526	1766	85	445
7755	5	1	165	476	946	1256	2022	2478	1759	40	470
7761	5	1	183	510	978	1393	2237	2625	1916	84	541
7768	5	1	159	458	982	1397	2210	2678	1883	53	533
7771	5	1	155	448	923	1322	2102	2293	1632	75	409
7778	5	1	130	328	708	1097	2054	2653	1848	63	521
7790	5	1	141	324	703	1052	1908	2508	1771	66	459
7793	5	1	169	513	1079	1494	2390	2777	1981	52	574
		average	154.3	425.0	887.0	1285.0	2114.3	2538.3	1808.8	51.3	503.3
		stdev	15.03	53.56	95.33	114.51	129.55	151.25	127.41	17.31	64.68
7501	2	2	139	418	857	1272	2076	2569	1788	56	514
7506	2	2	161	459	970	1432	2294	2892	2032	44	579
7512	2	2	156	440	865	1228	2033	2602	1836	52	533
7515	2	2	141	422	934	1391	2309	2845	2017	55	581
7517	2	2	149	382	839	1250	2154	2738	1940	42	573
7520	2	2	136	368	773	1112	1845	2314	1609	33	436
7522	2	2	145	431	935	1400	2264	2476	1799	38	518
7524	2	2	174	404	786	1138	1906	2375	1720	49	485
7526	2	2	148	428	886	1272	2010	2369	1642	35	476
7529	2	2	145	409	865	1308	2176	2700	1908	47	525
7532	2	2	171	457	920	1284	2119	2626	1839	69	497
7537	2	2	157	406	878	1281	2074				
7539	2	2	126	365	703	1056	1809	2235	1494	33	380
7569	2	2	155	452	932	1246	1910	2382	1685	66	447
7584	2	2	167	397	842	1248	2004	2249	1621	48	444
7586	2	2	160	422	872	1331	2233	2822	1917	49	537
7592	2	2	159	430	842	1213	1977	2478	1771	55	471
7594	2	2	128	350	740	1147	2038	2600	1801	44	474
7598	2	2	190	512	1009	1418	2423	2757	1961	52	552
		average	153.0	418.5	865.7	1264.6	2087.1	2557.2	1798.9	48.2	501.2
		stdev	16.09	38.16	77.44	103.92	166.93	208.20	149.96	10.14	54.87

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animal	pen	treatment	BW 7	BW 14	BW 21	BW 26	BW 35	BW 42	carcass	abdominal fat	breast
7204	3	2	161	456	947	1414	2262	2638	1836	37	523
7211	3	2	209	511	966	1379	2192	2567	1864	53	532
7219	3	2	170	454	934	1320	2240	2708	1935	45	570
7224	3	2	140	375	802	1177	1972	2446	1772	38	497
7225	3	2	156	448	942	1378	2175	2428	1764	67	449
7231	3	2	159	392	731	986	1439	1776	1238	14	384
7232	3	2	133	360	772	1146	2072	2442	1740	50	521
7235	3	2	145	422	901	1333	2209	2372	1739	52	500
7244	3	2	182	495	1030	1450	2180	2537	1828	36	519
7250	3	2	184	498	988	1406	2149	2242	1651	51	446
7252	3	2	153	442	942	1326	2131	2614	1872	56	550
7268	3	2	134	394	820	1224	2129	2709	1934	49	572
7273	3	2	146	386	833	1249	2078	2201	1583	37	405
7275	3	2	154	396	814	1236	2136	2685	1890	36	612
7277	3	2	160	369	806	1341	2178	2349	1721	50	496
7278	3	2	183	454	948	1342	2184	2338	1638	77	434
7297	3	2	184	466	954	1368	2111	2430	1704	47	446
7300	3	2	168	407	819	1225	2091	2603	1764	54	453
7546	3	2	161	420	872	1268	2156	2443	1695	39	462
7577	3	2	143	454	926	1241	2153	2630	1815	66	502
		average	161.3	430.0	887.4	1290.5	2111.9	2457.9	1749.2	47.7	493.7
		stdev	19.63	44.57	81.95	109.43	170.87	219.61	154.36	13.69	58.77
7705	6	2	137	348	787	1207	2061	2433	1753	41	497
7708	6	2	137	427	946	1408	2250	2718	1935	53	529
7711	6	2	160	420	798	1152	1885	2315	1664	40	506
7712	6	2	138	374	729	1126	1945	2450	1797	68	515
7725	6	2	141	368	702	726	1486	1999	1328	55	336
7730	6	2	119	284	560	880	1581	2321	1585	48	474
7735	6	2	178	505	1009	1430	2264	2817	1982	45	608
7738	6	2	147	433	829	1266	2262	2859	2026	76	558
7743	6	2	116	324	692	1078	1850	2367	1665	29	481
7746	6	2	183	497	968	1392	2102	2204	1621	69	412
7757	6	2	180	494	998	1398	2130	2631	1818	48	513
7759	6	2	138	411	886	1358	2388	2875	2038	46	571
7760	6	2	156	404	720	1004	1758	2366	1650	51	454
7762	6	2	156	456	998	1453	2312	2906	2064	62	618
7764	6	2	154	457	963	1379	2163	2457	1809	82	454
7777	6	2	184	461	939	1386	2228	2743	1974	55	516
7779	6	2	143	425	839	1241	2063	2433	1789	48	489
7785	6	2	122	325	756	1164	1929	2358	1647	47	463
7789	6	2	148	447	897	1328	2114	2626	1835	40	513
7792	6	2	139	435	940	1406	2306	2857	2032	46	576
		average	148.8	414.8	847.8	1239.1	2053.9	2536.8	1800.6	52.5	504.2
		stdev	20.36	61.00	126.28	198.89	246.34	256.38	192.01	13.12	65.91