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**Trademarks and Registration**

X™ indicates trademarked of product mentioned
X* indicates product is registered in terms of Act 36/1947

BIORED Reg no. V 20924
BIOSIN Reg no. G 1114
BIOSTROSIN Reg no. V 12928

**SIMILARITIES AND DEFINITIONS**

BIORED: A natural bioflavonol antioxidant
BIOSIN/BIOSTROSIN (similar products): A probiotic suspension consisting of a broad spectrum of Lactic Acid producing organisms.
NANDREA HEALTH PRODUCTS has over the last 31 years developed biological products for various animal species, with the aim to optimize production and health at reduced costs and improved net profit with “green” products. These products are biological in origin and thus natural. They are furthermore not genetically manipulated and are completely safe to use, even at much higher intakes than prescribed. The company has a dedicated research program and are collaborating with various universities in their research efforts. The results of these research projects will be reported as it becomes available and will be published in reputable peer review scientific journals.

1. BROILERS
Studies on commercial farms as well as at various universities, confirmed that:
A. In terms of average mass gain, feed conversion and drip loss there is no statistically significant difference between broilers receiving a diet containing 50% of the industry standard inclusion of vitamin A and E and 200g per ton Biored™ and broilers receiving a diet with the industry standard inclusion of vitamin A and E. In terms of these results 50% of industry standard inclusion of vitamin A and E can be replaced with Biored™. Biored™ also negates the need for the inclusion of any other antioxidant in the feed. (SEE ATTACHMENT C: UFS WIDD, as well as ATTACHMENT D: BROILERS UP)

B. BIOSIN™, a liquid probiotic, consisting of the BIOREM® culture, was included in various studies in broilers, resulting in improvement on survival and weight gain. (SEE ATTACHMENT E)

2. LAYERS
BIOREM™ and BIOSIN™ were used from week 44, as an aid to increase survivability. This age was chosen as on this farm historically mortalities starts to increase. The observation reported here are compared with the average performance for the specific breed and farm. Culling usually take place at week 75, as a result of production loss due to mortalities. BIOSIN™ is known to improve the health of lambs on farms where antibiotic resistant E. coli was identified, as well as in various other infectious diseases. Inhibition in vitro indicated antagonism against various pathogens. (Find laboratory results as ATTACHMENT B)
For hens treated with BIOREM™ and BIOREM™ a lower mortality rate was than the other houses throughout the production cycle, resulting in more hens that could produce eggs. Looking at the eggs per hen graph below, the number of eggs laid per hen was higher over the production cycle than the breed standard estimation although these houses were not fully environmentally controlled. At culling on week 85 it was still, in terms of production, economically viable to keep the layers. (SEE ATTACHMENT A, SURVIVABILITY GRAPH).
The improved survival rate and the resultant sustained egg production have a significant beneficial financial impact for egg producers. (Find report on Layers as ATTACHMENT A)

3. PROBIOTICS
The BIOREM®-culture consists of a broad spectrum of Lactic Acid Producing organisms, with antagonism against certain pathogens in the digestive system of man and animals. Several in-vivo (ATTACHMENT C) as well as in-vitro trials (ATTACHMENT B) have proofed efficacy of the culture in ostriches (ATTACHMENT F) and poultry (ATTACHMENT A – layers, and C - broilers). Trail results are also available for monogastric animals (pigs) as well as ruminants.

Antibiotic-resistance pathogens: The BIOREM®-culture has been used over a large area where antibiotic-resistance E. coli caused epidemic mortalities in lambs. Clear indications were found that BIOREM® was the only treatment that could decrease mortalities as result of E. coli infections. At certain poultry farms, where E. coli was suspected, but not confirmed, improvement on various parameters were obtained.

4. NATURAL ANTIOXIDANTS
BIORED* is a natural plant extract known as a polyphenolic antioxidant, that consists of Monomeric flavonoids and Polymeric anthocyanidins.
It has a vitamin sparing effect on Vitamins A, E and C; The antioxidant activity regenerates both naturally occurring and added Retinol (Vit A), Alpha-tocopherol (Vit E), as well as Ascorbic Acid (Vit C) in the feed supplied to the animal. This increased availability of Vitamins A, E and C is attributed to Biored’s ability to scavenge free radicals; When compared to synthetic anti-oxidants, Biored performs competitively and increases the digestibility of neutral detergent fibre (NDF) in the gastrointestinal tract; Increases ADG; Lowers Drip Loss on meat cuts; Increases hair, nail, hoof and feather growth; Increases collagen strength to prevent tearing of intestines and skin; Stimulates lactate consuming organisms reducing risk of acidosis; IT IS SCIENTIFICALLY PROVEN TO ACT, BOTH IN-VITRO AND IN-VIVO, AS AN ANTIOXIDANT AS WELL AS AN ANTIRADICAL AGENT.

PROBIOTICS AND NATURAL ANTIOXIDANTS
The use of probiotics and bioflavonol antioxidant in commercial trials were very promising in terms of improvement of mortality, growth, and cost savings. Several experimental and commercial trials were conducted in several animal species, and the advantage of both products were investigated on the basis of performance, as well as analytically.
5. ATTACHMENT A

Use of Biored™ (anti-oxidant) and Biosin™ (Pro-biotic) supplements in an open-sided layer house at Nantes Estate (Windmeul) outside Paarl
07/01/2019

BACKGROUND

Due to the bird flu epidemic in 2018 and the resultant widespread mortalities of layers and their parent stock, industry is experiencing an inadequate supply of pullets as replacement stock. Profits are further under pressure due to high feed and input costs and small margins. This makes it important to minimize the loss hens to pathogenic organisms such as Salmonella, Coccidiosis, E. coli, Mycoplasma galiarium. Egg producer, Nantes, investigated strategies to decrease the risk of losing hens unnecessarily.

Decrease mortalities were reported for broilers, ostriches and other ruminant livestock using Biored™ and Biosin™. The antioxidant, Biored™ (200g/ton) and the probiotic, Biosin™, were added to the feed and the water of layers to obtain an indication if these two products could reduce mortalities, improve flock and individual bird health and increase survivability. If observed this would increase the average period that hens can be kept in the flock and allow a longer production cycle.

The laying hens received Biored™ and Biosin™ from the age of 44 weeks until they were culled in week 86. The test house had 18,106 birds at initiation and 16,711 at culling.

Similarities between the houses:
- Same breed – Lohmann Light Brown
- Houses are located next to each other and on the same biosecurity site
- All houses are open-sided houses
- All houses had the same vaccination programme
- Most pronounced differences between the houses:
- Houses 2 – 6 with which the test house is compared to were all placed between 03/03/2016 and 01/12/2016 at age of 18 weeks. The test house was placed on 17/08/2017 at age of 18 weeks.
- The different houses had different managers with different management styles.
- Different feed companies were used during the production cycle of the comparison houses. The test house had the same feed company throughout the production cycle.
- Different months of the year at the same age between flocks.
- In terms of the survivability graph below (yellow line), the mortalities observed in the test house was lower than the other houses throughout the production cycle, resulting in more hens that could produce eggs. Looking at the eggs per hen graph below, the number of eggs laid per hen was higher over the production cycle than the breed standard estimation although these houses were not fully environmentally controlled as would be the case for the breed standard estimation. These two factors resulted in more eggs being produced in the test house than previously experienced by other flocks under similar housing conditions. It is further interesting to note that the sharp increase in mortality observed in houses 2, 3 and 6 between weeks 60 and 70 were not observed in the test house. In terms of this observation it is not clear what individual contribution Biored™ and Biosin™ made to the improved survivability of the layers.

The results discussed are a summary of observations and production records and should not be confused with a scientific study under controlled conditions. This preliminary observation run was done to determine if a full-scale scientific study was justifiable and the results indicated that these products could very well be tested under controlled conditions to scientifically prove their effectiveness.

Report compiled by Dr Mariaan Viljoen and Mr Craig Shepstone
6. ATTACHMENT B

BIOSTROSIN*: Pathogen-inhibition In vitro Antimicrobial effect determination

BACKGROUND
The BIOREM-culture consists of a broad spectrum of Lactic Acid Producing organisms, with antagonism against certain pathogens in the digestive system of man and animal. Several in-vivo trials have been proofed efficacy of the culture in ruminants as well as monogastric animals, and in pigeons, ostriches and poultry.

The aim of this study is to demonstrate inhibition of poultry-specific pathogens by aids of agar plate inhibition zones. The results confirm previous similar tests where inhibition of individual strains were demonstrated against pathogenic E.coli. In fact, synergism has also been demonstrated by measuring of inhibition zones of the individual strains, and compared with the pooled stains, known as the BIOREM-culture commercially available as the product BIOSTROSIN*. W.H. Holzapfel (Prof. Microbiology) described in his statement on Different Issues regarding Probiotic Microorganisms that L. Casei-group are actually phylogenetically related of three different independent species L.casei, L. paracasei, and L. rhamnosus. These organisms are al included in the BIOREM-culture. He also stated that the origin of organisms used as probiotic does not necessary originate from the host they are applied. These statement is supported by Mitsuaka, 1992, personnel communication.

Also important is the importance of growth medium after cultivation of liquid probiotics which contains beneficial metabolic products and exert antimicrobial effects against pathogens. (Personal communication: P. Steyn, professor in Microbiology, Univ. Pretoria, 1990 — 2013)

INVESTIGATERS
J Jacobs, Deltamune
PWN Groenewald, Biorem Biological Products
Prof. W.H. Holzapfel, Biorem Biological Products

SHELF LIFE AND DOSAGE
Shelf life for the liquid product has been proofed to be at least 6 months after preparation.

The dried product (commercially known as the product BIOREM, reg. No. G985, act 36/1947) have a shelf life of at least 5 years, when stored away from light, cool and in sealed container to prevent it from loosing moisture.

Dosage during trials were done at an average bacterial count of $1 \times 10^7$.

The average bacterial count of the liquid products after incubation is $1 \times 10^8$. In trials where the feed has been enriched with the probiotic culture, bacterial counts were average $1 \times 10^9$.

It is thus clear that quantity is relative to efficacy, but the route and time of administration, specific characteristics of probiotic strains and target specie determines the outcome, as it is life organisms which multiply and grow within the digestive system.

Acute treatment will need a substantial amount of microbes, while preventative treatment need smaller amounts over a longer period.

INHIBITION OF PATHOGENS ON AGAR PLATES
Three cultures have been supplied:
Lactic Culture: a Lactic Acid Bacteria culture commercially available as reference positive control.
Biorem culture as test culture.
B. laterosporus culture as positive reference, a well known probiotic international.

TEST STRAINS
Well known pathogens in poultry were used as test strains:
E. Coli
Salmonella serovar typhimurium
Salmonella serovar enteritidis
Campylobacter jejuni

INHIBITION TEST ON AGAR PLATES
Test lay out is attached as REPORT from page 10

Procedure:
The following cultures were used to determine whether or not Biorem will have an antimicrobial effect against these organisms: E. coli
Salmonella serovar Typhimurium
Salmonella serovar Enteritidis
Campylobacter jejuni
All test isolates were grown overnight to ensure they were in the logarithmic growth phase. The Biorem cultures "A" and "B" were also grown overnight in MRS broth. Biorem culture C was grown overnight in nutrient broth.

After incubation each test isolate was suspended to a cell density of 108cfu/ml in Tryptone Soy broth.

100111 of each suspension was spread onto the surface of a Plate count agar plate. Three holes of 9mm were punched into each agar plate.

100111 of each of the Biorem cultures were placed in a separate hole. After inoculation the plates were incubated at 37°C for 24 hours.

Results:
All three cultures showed an antimicrobial effect against all of the test organisms. Refer to the pictures below:

CONCLUSION
The BIOREM-culture has been proofed antagonistic against pathogens as indicated, usually associated with mortalities in poultry.

LITERATURE


7. ATTACHMENT C

(UFS WIDD: Effect of suboptimal vitamin inclusions in rations with and without BIORED: BIOFLAVONOIDIC ANTIOXIDANT)

1. BIORED: BIOFLAVONOIDIC ANTIOXIDANT

*BIORED is a natural water extraction from a legume with a very high ability of scavenging free-radicals and chelating heavy metals. It is also known as a polyphenolic antioxidant.

Free radicals have a negative effect on the body by binding to protein, especially surface protein such as epithelial cells, but also to others that cause tissue damage, protein synthesis slows down, enzymatic activity is disturbed, fats oxidize and become rancid, etc. Especially with modern farming
practices where high production systems used are the trend of free radical high, with negative effect on profitability.

* BIORED primarily acts as antioxidant with a special high scavenging effect, ranging between 600 000 and 800 000 trollox units per 100 grams, also known as the ORAC value. That's almost double the value of known grape seed extracts.

It has been proved that with 0.02% m/m inclusion of BIORED, Vitamin A and E levels can be reduced with up to 50% of recommended ratio in feed. This leads to cost savings on the sparing of vitamin A and E inclusion in feed.

A summary of results of suboptimal vitamin inclusions with or without inclusion of BIORED at different inclusion rates, when compared with optimal vitamin inclusion:

2. THE EFFECT OF BIOFLAVONOIDS ON PRODUCTION OF BROILERS

2.1. Mortality rate in broilers
Feeding diet with suboptimal Vit A and E levels had a cumulative mortality rate of 20.66%. The addition of BIORED at to a diet with suboptimal Vit A and E levels slowed down the mortality rate significant.

2.2. Growth
There was no significant difference between the control group (Optimal inclusion of vitamin levels) and groups where BIORED was included in the diet with sub optimal vitamin levels.

2.3. Feed conversion
Feed conversion in broilers on day 40 was for the Control group was 1.82, Suboptimal Vitamin inclusion group 1.83, Suboptimal Vitamin inclusion group with BIORED was 1.81, and the normal Vitamin inclusion group with BIORED 1.80. There was no significant differences (p< 0.05) between treatment groups.

2.4. Life mass
No significant differences (p< 0.05) between treatment groups were seen.

2.5. Vitamin status
Ruminants
Alpha-tocopherol: Liver Alpha-tocopherol concentration of groups with Low Vitamin Status plus BIORED, as well as Normal Vitamin Status plus BIORED, was significantly (p< 0.05) higher, 103.9% than the Low Vitamin Status Group without BIORED, and 50.6% higher than the Normal Vitamin Status group without BIORED.

Broilers
A trail on layers was done with the treated group receiving a "normal" diet and recommended vitamin inclusion, while the control group received the same ration with 0.07% m/m BIORED supplementation.

BIORED supplementation protected vitamins from in vivo oxidation in broilers. Alpha-tocopherol concentration in the liver was 40% higher than the control and retinol 97%. It was concluded that BIORED has a vitamin sparing effect, and less vitamins can be included in feed.

2.6. Retinol
Liver Retinol status: A trail was done with an 50% retinol inclusion and 0.07% m/m BIORED, 50% retinol inclusion without BIORED and a group fed with normal vitamin status without BIORED and without BIORED. Although there were no significant differences (p< 0.05) between dietary treatments, the Low Vitamin Status Group with BIORED, and the Normal Vitamin Status group with BIORED was respectively 5.98% and 15.24% higher than the diet groups without BIORED inclusion.

2.7. Broiler liver mass
Liver mass of the Low Vitamin Status Group with BIORED, and the Normal Vitamin Status group with BIORED was 16.6% and 15.3% higher than the control diet group. This correlated well with published data by Takaos et al, (1977) and Sonnenbichler & Zetl, (1986), who showed that flavonoids have a positive effect on protein synthesis by stimulating ribosomal RNA synthesis. Diagnosis by a poultry veterinarian and pathologist excluded the possibility of flavonoid toxicity. In fact, this is part of the explanation why an increase in antioxidative vitamin status is found when BIORED has been added to rations.

2.8. Conclusion on broiler trials
These data showed that BIORED-inclusion could replace at least 6000 – 6500 IU/Kg vitamin A, and 15 – 20 IU/Kg vitamin E in the diet.

8. ATTACHMENT D

BROILERS UP

In this trial 8 different dietary treatments were used with the aim to confirm the efficacy and optimal inclusion level of BIORED in Broilers. The different treatments are set out in Table 3.

Table 3. The different treatments used to determine efficacy and optimal inclusion levels for broilers.

<table>
<thead>
<tr>
<th>Treatment name</th>
<th>Biored inclusion level (g/ton)</th>
<th>Vit A &amp; E inclusion level (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Positive control)</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2 (Negative control)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>150</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>150</td>
<td>50</td>
</tr>
<tr>
<td>8</td>
<td>200</td>
<td>50</td>
</tr>
</tbody>
</table>
As can be observed in table 5 there were no significant differences in the carcass weight between the positive control group and the groups that received 50% of the supplementary vitamin A and E and 150 or 200g Bioredo per ton of feed. The Negative Control group and Treatment 3 (0% supplementary vitamin A & E) had significantly lower carcass weights than the Positive Control group.

Drip loss, cooking loss and shear force were measured for the different treatments in the left breast.

Table 6. Cooking loss, drip loss and shear force measurements in left breast meat.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drip loss</th>
<th>Cooking loss</th>
<th>Shear Force</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (+ control)</td>
<td>2.11bc</td>
<td>30.58</td>
<td>44.21 abc</td>
</tr>
<tr>
<td>2 (- control)</td>
<td>2.43a</td>
<td>28.71</td>
<td>40.74 a</td>
</tr>
<tr>
<td>3</td>
<td>2.21b</td>
<td>27.94</td>
<td>42.76 aic</td>
</tr>
<tr>
<td>4</td>
<td>2.15bc</td>
<td>28.17</td>
<td>49.44 a</td>
</tr>
<tr>
<td>5</td>
<td>2.26a</td>
<td>28.85</td>
<td>42.65 a</td>
</tr>
<tr>
<td>6</td>
<td>1.75ac</td>
<td>28.04</td>
<td>43.85 a</td>
</tr>
<tr>
<td>7</td>
<td>2.37ac</td>
<td>29.1</td>
<td>41.85 a</td>
</tr>
<tr>
<td>8</td>
<td>1.63ac</td>
<td>28.73</td>
<td>45.64 ac</td>
</tr>
<tr>
<td>SE</td>
<td>0.251</td>
<td>1.03</td>
<td>2.64</td>
</tr>
<tr>
<td>R 2</td>
<td>0.525</td>
<td>0.525</td>
<td>0.457</td>
</tr>
</tbody>
</table>

abcd Column means with the same subscripts do not differ significantly (P>0.05)

The drip loss of breast meat did not differ significantly between treatments, except for Treatment 8 (200g Bioredo, 50% vitamin A and E) which had the lowest drip loss. This is economically important as it is a major factor in carcass weight loss and it might also improve water retention prior to freezing.

No statistically significant differences were found in loss of moisture during cooking of the breast meat between treatments. Treatment 4 had the highest shear force strength and differed significantly from Treatments 2 and 7. The lowest shear force strength was noted for the Negative Control (P>0.05).

Lipid oxidation: Lipid peroxidation determines the degree of rancidity of fats and is therefore an important factor in determining shelf life. No significant differences in the lipid peroxidation of the chicken thigh meat was observed between groups 1, 6, 7 and 8 (the groups receiving 100% of vitamin A and E and the groups receiving 50% of the vitamin A and E and between 100 and 200g per ton Bioredo), using TBARS testing. The control group showed a significantly higher degree of lipid peroxidation.

3. SUGGESTED ADMINISTRATION PROGRAM

BI OSTROSIN: Apply in drinking water at 1 liter per 1000 as follows (recommended route):

Day 1 to 3
Then 2 times a week until slaughter.

ALTERNATIVELY: Spray 1 liter of product per ton feed. No antibiotic inclusion.

Reduce vitamin A and E inclusion with 50%.

As can be observed in table 5 there were no significant difference in the carcass weight between the positive control and the different treatments.

In terms of body weight growth the feeding of 50% of the prescribed vitamin A & E, but with Bioredo in their feed added at increasing levels, did not differ significantly from the Positive Control. In terms of body weight the feeding of 50% of the prescribed vitamin A and E supplemented with between 100g and 200g per ton of Bioredo renders the same growth rate.

In table 5 below the carcass traits for the different treatments are given.

Table 5. The carcass and portion weights of the carcasses for the different treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Carcass Weight (kg)</th>
<th>Right Thigh (g)</th>
<th>Right Breast (g)</th>
<th>Right Thigh (% of carcass weight)</th>
<th>Right Breast (% of carcass weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (+cont)</td>
<td>1.81a</td>
<td>141.71bc</td>
<td>255.29bc</td>
<td>7.83</td>
<td>14.10</td>
</tr>
<tr>
<td>2 (-cont)</td>
<td>1.66a</td>
<td>129.54bc</td>
<td>236.42bc</td>
<td>7.8</td>
<td>14.24</td>
</tr>
<tr>
<td>3</td>
<td>1.73bc</td>
<td>135.88bc</td>
<td>246.50bc</td>
<td>7.85</td>
<td>14.25</td>
</tr>
<tr>
<td>4</td>
<td>1.79bc</td>
<td>138.96bc</td>
<td>249.21bc</td>
<td>7.76</td>
<td>13.92</td>
</tr>
<tr>
<td>5</td>
<td>1.79bc</td>
<td>140.83bc</td>
<td>246.00bc</td>
<td>7.87</td>
<td>13.74</td>
</tr>
<tr>
<td>6</td>
<td>1.75bc</td>
<td>133.13bc</td>
<td>247.67bc</td>
<td>7.61</td>
<td>14.15</td>
</tr>
<tr>
<td>7</td>
<td>1.79bc</td>
<td>138.27bc</td>
<td>244.83bc</td>
<td>7.72</td>
<td>13.68</td>
</tr>
<tr>
<td>8</td>
<td>1.78bc</td>
<td>141.33bc</td>
<td>253.33bc</td>
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<td>14.23</td>
</tr>
<tr>
<td>SE</td>
<td>0.025</td>
<td>3.34</td>
<td>6.38</td>
<td>0.10</td>
<td>0.23</td>
</tr>
<tr>
<td>R 2</td>
<td>0.525</td>
<td>0.525</td>
<td>0.457</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

abcd Column means with the same subscripts do not differ significantly (P>0.05)
9. ATTACHMENT E

THE BIOREM CULTURE IN POULTRY: FARM TRAILS

1. TRIALS

Trials were done under various conditions, and the conclusion was that there was not only saving on production costs, but it also had an improvement on growth performance.

In the first trial, the initial problem was bacterial infection which led to increase in mortalities over a long period. Antibiotic treatment had to be increased over time to get the necessary effect as a result of increasing resistance of pathogens. It also led to increase in cost of treatment.

Treatment with the BIOREM-CULTURE has been implemented. Treatment was through drinking water.

TRIAL 1 Tasty/Modderrivier

The farm consisted of 12 rearing houses for 10 000 birds each.

TREATMENT
The broilers were treated through the water supply system at a rate of 1 liter per 1000 liters of drinking water.

Frequencies of treatment were:
Day 1 to 3
Day 7, 14, 21, 28 and 35.
No antibiotics were included in rations mixed.

MEASUREMENT
As there was no untreated control group, it was not possible to compare efficiency of the probiotic. However, mortalities were between 2,5 and 4,5 %, which compared well with the proceeding period when antibiotics were included in rations. Carcass weight was satisfactory, and feed conversion were normal and even improved for this farm from an average of 2,01 to 1,9 Kg feed per Kg carcass weight gained.

RESULTS
Although the cost of probiotics were substantially more than antibiotic treatment of feed, as well as a slight increase on handling costs, the farm experienced a much more stable production rate and meat could be submitted to consumers as healthy with no fear of transmitting antibiotic resistant pathogens.

The little improvement in feed conversion resulted in a sparing of 19,8 tons of feed. (361 tons needed to produce 1 full run of poultry meat in 12 houses of 10 000 broilers each, compared to 342 tons to produce the same amount after implementation of the probiotic)

CONCLUSION
The improvement in production thus covers the extra cost on the implementation of the probiotic treatment and also resulted in a net income improvement.

Healthier poultry meat was produced which contributed to public health.

TRIAL 2 Vaalharts

Trials were done on a small commercial broiler farm to determine the effect on profitability and consistency of production in small production units.

MATERIALS AND METHODS
On a specific broiler farm, where mortalities fluctuated from “normal” to severe, without any known reason, a Lactic Acid Culture, commercially known as BIOSTROSIN*, was administered to one house through the drinking water, and the other one was left as untreated control. The 2 houses were selected for similar floor space; alongside each other; the external conditions and history of growth and mortalities.

The trial was done during late winter (August), and repeated during early spring (October).

It was a commercial trial, and average weight gain and mortalities were the only two measurements. The ration supplied was mixed on the farm by the owner, and the recipe had been used for several years with good results. No antibiotic was included.

The floor space was sufficient for the 5000 chicks, as well as regulated temperature and ventilation. All factors remained the same.

TREATMENT
House no. 1 received treatment through the water system. Water was tested for purity, and no chlorinating was done. The BIOREM-culture (BIOSTROSIN) counted average 1,6 X 10⁸ per ml, and was diluted 1 per 1000 litre.

Treatment of drinking water was done on day 1, 2, 3, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37.

House no. 2 was left untreated.

Weighing was done on day 14, 21, 28 and 35 on a sample basis representing an average of the total house.

Mortalities were monitored weekly.

Birds were slaughtered on day 38.

RESULTS
LATE WINTER TRIAL
House no. 1 Biorem-Culture Treated Group (BIOSTROSIN*)

<table>
<thead>
<tr>
<th>Day</th>
<th>Mortalities</th>
<th>Mort Accum</th>
<th>Avg Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>24</td>
<td>-</td>
<td>239</td>
</tr>
<tr>
<td>14</td>
<td>21</td>
<td>45</td>
<td>456</td>
</tr>
<tr>
<td>21</td>
<td>10</td>
<td>55</td>
<td>873</td>
</tr>
<tr>
<td>28</td>
<td>37</td>
<td>92</td>
<td>1448</td>
</tr>
<tr>
<td>35</td>
<td>51</td>
<td>143</td>
<td>7033</td>
</tr>
</tbody>
</table>

Percentage of mortalities: 2,86 %
Total meat produced: 1,448 x (5000 – 143) = 7033 Kg

House no. 2 Untreated Control Group

<table>
<thead>
<tr>
<th>Day</th>
<th>Mortalities</th>
<th>Mort Accum</th>
<th>Avg Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>32</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>27</td>
<td>59</td>
<td>224</td>
</tr>
<tr>
<td>21</td>
<td>63</td>
<td>122</td>
<td>458</td>
</tr>
<tr>
<td>28</td>
<td>62</td>
<td>184</td>
<td>853</td>
</tr>
<tr>
<td>35</td>
<td>41</td>
<td>225</td>
<td>1368</td>
</tr>
</tbody>
</table>
**Percentage of mortalities:** 5.38 %  
**Total meat produced:** $1,368 \times (5000 - 225) = 6532$ kg  
**Improvement on meat production:** 501 Kg

**EARLY SPRING TRIAL**  
**House no. 1 Biorem-Culture Treated Group (BIOSTROSIN*)**

<table>
<thead>
<tr>
<th>Day</th>
<th>Mortalities</th>
<th>Mort Accum</th>
<th>Avg Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>35</td>
<td>-</td>
<td>228</td>
</tr>
<tr>
<td>14</td>
<td>27</td>
<td>62</td>
<td>442</td>
</tr>
<tr>
<td>21</td>
<td>15</td>
<td>77</td>
<td>898</td>
</tr>
<tr>
<td>28</td>
<td>23</td>
<td>100</td>
<td>1237</td>
</tr>
</tbody>
</table>

**Percentage of mortalities:** 2.74 %  
**Total meat production:** $1,237 \times (5000 - 140) = 6011.8$ Kg  
**Improvement on meat production:** 176 Kg

**House no. 2 Untreated Control Group**

<table>
<thead>
<tr>
<th>Day</th>
<th>Mortalities</th>
<th>Mort Accum</th>
<th>Avg Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>38</td>
<td>-</td>
<td>225</td>
</tr>
<tr>
<td>14</td>
<td>26</td>
<td>64</td>
<td>418</td>
</tr>
<tr>
<td>21</td>
<td>25</td>
<td>89</td>
<td>808</td>
</tr>
<tr>
<td>28</td>
<td>28</td>
<td>117</td>
<td>1204</td>
</tr>
</tbody>
</table>

**Percentage of mortalities:** 3.0 %  
**Total meat production:** $1,204 \times (5000 - 153) = 5835.8$ Kg  
**Improvement on meat production:** 176 Kg

**TRIAL 3**  
A trial was conducted on a smaller poultry farm to investigate profitability when a probiotic is used.  
Only mass gain and mortalities were measured.

**MATERIALS AND METHODS:**  
**TRIAL ANIMALS**  
1500 Chickens were randomized divided into 2 groups without taking genders in account. Gender was taken into account on day of slaughter.  
Both groups were kept in the same building to keep circumstances the same.

**FEEDING**  
Commercial rations were fed to trial groups ad lib as prescribed by the manufacturer.  
The average of the starting mass was calculated, as well as end mass at slaughter.

**HOUSING**  
The house ensured enough space for 2000 broilers.  
Solid partitions were used to keep the 2 groups separated without any cross contamination.  
Standard feeding and water equipment were used.  
The water supply was separated for the 2 groups to ensure that only the treated group received treatment through the drinking water.  
The house had concrete floors and walls and there was sufficient ventilation.

**MONITORING**  
Mass increase was monitored, calculated on day 1 and day 35, as well as mortalities over the trial period.

**TREATMENT.**  
Drinking water was treated with BISTROSIN, freshly supplied by BBP, mixed at a rate of 1:1000.  
The calculated bacterial count was $1.6 \times 10^8$ of the culture which had been diluted to 1:1000 in drinking water.  
Each broiler received an average of 11 ml product over the 5 week period.  
A total of 8 liter product has been used.

**RESULTS**

<table>
<thead>
<tr>
<th>N</th>
<th>BIOSTROSIN</th>
<th>CONTROLL</th>
<th>Dosage in ml / 35 days</th>
<th>Total of product in ℓ</th>
<th>Mortalities %</th>
<th>Mass increase 35 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>700</td>
<td>11</td>
<td>0</td>
<td>8</td>
<td>2.9</td>
<td>5.6</td>
<td>1.76</td>
</tr>
<tr>
<td>800</td>
<td>1.64</td>
<td>1.41</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**CONCLUSION**  
The administering of BISTROSIN seems to improve weight gain as well as decrease mortality rate in broilers over 35 days when treated through drinking water, in comparison with an untreated control group.

**2. SUMMARY**  
Two independent laboratory- in vivo trials were done as well as 3 commercial broiler trials at 3 different farms. They differ in quantities raised, farming methods, feeding and farming conditions. The in vivo trial results were supported by results of the commercial farms. Mortalities decreased, while growth and feed conversion improved. The improvement on the commercial value made the use of probiotics as described economical justifiable.

**RECOMMENDED TREATMENTS**

*BI-OSTROSIN: Administer through drinking water at 1 per 1000 litre as follow: Day 1 to 3, dosage 0.5 ml per head of product. Thereafter every second week, 0.5 ml per head, until slaughter.*

**10. ATTACHMENT F**

**OSTRICH FEEDLOT TREATMENT REPORT**  
It is well known that ostriches are very tensed animals, and therefore become much more stressed than other species may become during stress-causing situations. Stress in ostriches manifest in loss of appetite, resulting in loss of weight, reduced immunity, loss of condition, lameliness, and eventually getting sick and mortalities may occur. This usually happens over 7 to 10 days post stressed.

A group of 100 ostriches, with mass varying between 45 and 60 Kg, was received in a feedlot, coming from various farms, traveling over a distance more than 100 Km. To minimize stress further, the group was divided randomly in an untreated
control and a treated group. 75 Birds were treated with 10ml BIOSIN* orally once on day 2 of arrival. Both groups were weighed individually on day of treatment, as well as day 10 post treatment. Average mass gain for each group was calculated.

In this trial, it was clear indicated that 72.09 % of the treated group gained mass of average 4.2Kg over a period of 10 days, while 27.09 % of the same group lost mass. In the untreated group, 56.14% birds gained an average of 2.25 Kg over this period, while 43.85% lost weight.

**OSTRICH FEEDLOT RESULTS**

<table>
<thead>
<tr>
<th></th>
<th>Treated</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>57</td>
<td>43</td>
</tr>
<tr>
<td>% of total increased in weight</td>
<td>72.09</td>
<td>56.14</td>
</tr>
<tr>
<td>% of total decreased in weight</td>
<td>27.09</td>
<td>43.85</td>
</tr>
<tr>
<td>Average mass gain (Kg)</td>
<td>4.2</td>
<td>2.25</td>
</tr>
</tbody>
</table>

Single dosage of 10ml Bi-ostrosin on day 1
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