Production of Antibiotic Free Chicken and *in-vivo* & *in-vitro* Evaluation of Atomes Novel Antibiotic Replacement Biotechnology, Novo Biotic ®, in Broiler Chicken Compared to Antibiotic Growth Promoters Avilamycin

Johnny Nehme^{1,4*}, Ihab Rishan², Fadi Dagher¹, Marwan Dagher⁴, Dory Dagher¹, Ziad El Sayed², Haitham El Nawame² and Walid Saleh^{3,4}

¹R&D Department of the Animal Healthcare Division, Atomes FD Inc, Canada

²SNAGRO s.a.r.l - Lebanon

³Consultant: Doctor in Veterinary Medicine (Immunology), Middle East, Africa, Europe

⁴Atomes F.D. Inc. Regional Office, Middle East, Africa, Europe & Asia

Submission: January 19, 2021; **Published:** February 22, 2021

***Corresponding author:** Johnny Nehme, R&D Department of the Animal Healthcare Division, Atomes F.D. Inc., Canada

Abstract

A Patented Novel Antibiotic Replacement Technology (ART) Novo Biotic ® (Multi-Strain spores forming Bacillus FD 777) was validated *in-vivo* & *in-vitro* based on its potentialities and capabilities of producing 15 complex of enzymes and more than 34 types of Lipopeptides (Bio-Antibiotics) in the production of broiler chicken. A total of 53'120 one-day-old ROSS 308-AP chicks were placed in 6 blocks and reared to 40 days. Control groups treated with Antibiotic Growth Promotor Avilamycin at 150g per Ton of feed, groups treated with Avilamycin at 150mg per ton of feed and Novo Biotic ® at 1L per 1000L of drinking water for 7 days and later 4 days per week till Day of Depletion (DOD between 38 and 40 days), and a group only treated with Novo Biotic ® at 1 L per 1000L of drinking water for 7 days and later 4 days per week till the DOD. The mean of the cumulative results of 1 to 40 days age revealed that birds treated with Novo Biotic ® with Avilamycin showed an FCR of 1.56 meanwhile the groups control treated only with AGP showed and FCR of 1.67. The treated group only with Novo Biotic ® showed an FCR at 1.53 compared to 1.67 of the control group. It's clear that the AGP is affecting the performance of the Bacillus Strains in the Novo Biotic ®. With the best FCR 1.53 obtained with only Novo Biotic ® vs AGP alone 1.67, along with the *in-vitro* studies against *Salmonella*, *E. Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* with an inhibition zones between 25mm to 30mm, the Novel Novo Biotic ® is considered as an "ART" unique in its category, by preventing challenges and promoting an optimum Broiler performance.

Keywords: Novo biotic ®; FCR; EPEF; Antibiotic growth promoters (AGP); Probiotic; Elisa

Introduction

Antibiotic growth promoters (AGPs) have been widely used to improve the performance of Broilers and the food conversion rates and they are well documented [1]. Antimicrobials are given to broiler chicken to control diseases such as necrotic enteritis caused by Clostridium species [2-6] in addition to Salmonella Species [7-9] and *E. Coli*, [10,11]. Antibiotics have started to fail. The rapid emergence of resistant bacteria is occurring worldwide, endangering the efficacy of antibiotics, which have transformed medicine and saved millions of lives [12,13]. Resistant bacteria

already causing more than 750,000 deaths every year and scientists did not introduce any new molecule since 1987. 106 million new cases/year are being seen and the consequences of total resistance would be devastating. This number is predicted to rise dramatically if radical actions are not taken. More than 60% of the populations in some areas carry multidrug-resistant bacteria in their normal bacterial flora. 214,000 newborns are estimated to die every year from blood infections (sepsis) caused by resistant bacteria - representing at least 30% of all sepsis deaths in newborns [14-22].

Antimicrobial resistance (AMR) is a global health threat and antimicrobial usage and AMR in animal production is one of its contributing sources [22,23]. Poultry is one of the most widespread types of meat consumed worldwide and are often raised under intensive conditions using large amounts of antimicrobials to prevent and to treat diseases, as well as for growth promotion [24]. Researchers estimate that the global average annual consumption of antibiotics per kilogram of chicken produced is 148 mg/kg [25]. Chicken harbors large proportion of Enterobacteriaceae resistant to aminosides in its digestive tract and tetracycline in its meat [26]. Another Canadian study [27] highlights the existence of different stereotypes of Salmonella, isolated from broiler farms, resistant and multi-resistant to antibiotics. In addition, antibiotic resistance in *Enterococci* [19], *Mycoplasma Gallisepticum* [28] and *Salmonella* spp. [29] isolated in broilers have been reported.

AGPs have been banned in more than 30 countries worldwide, mainly due to concerns about human health and the emergence of superbugs, or bacteria resistant to multiple types of antibiotics. Thirty-five countries have a veterinary prescription requirement [3,4,19]. The European Commission (EC) decided to ultimately ban (January 1, 2006) all commonly feed antibiotics used as growth promoters (EC Regulation No. 1831/20031) [30,31]. Finding

alternatives of AGP is now a viable solution to save the livestock sector. Probiotics have been proved to be the most preferred and effective alternative to antibiotics as a growth promoter and pathogens inhibitor in poultry industry [32-34]. In this study both *in-vitro* and *in-vivo* evaluation of a Patented Biotechnology based product originated 100% from biofermentation, Novo Biotic®, produced by Atomes FD. Inc. Canada was evaluated against an antibiotic growth promoter (AGP) Avilamycin.

Novo Biotic® is an innovation patented product that is used as 2 in 1 product:

1- Probiotic with a minimum of 1 billion CFU per ml of patented Bacillus FD777 multi-strains spore forming for its capability of producing more than 15 complexes of enzymes. Antibiotic Replacement Technology (ART) for its capability of producing more than 34+ types of bio antibiotics (Lipopeptides). All previous studies showed a complex of 34 Lipopeptides expressed genetically on the FD 777 strains such as: Macrolactin, Bacillomycin, Bacillaene, Fusaricidin, Difficidin, Bacitracin, Iturin, Tridecaptin, Bacilysin, Plipastatin, Surfactin, Teichuronic acid, Bacteriocin, Subtilin, Bacillibactin, Locillomycin, Kalimantacin, Myxovirescin, Plantathiazolicin, Citrulline, Plantazolicin, Bacitracin, Batumin, Nosperin and Fengycin (Table 1).

Table 1: Comparative table between regular antibiotic and Novel ART Novo Biotic®

Regular Antibiotic	Novo Biotic®
Find anywhere	Patented
Danger: residues in the Human body	No residues in the human body
Type: Synthetic molecule	Type: 100% Bio/Organic molecule
Technology: Single Molecule	34 Molecules (More Efficient) / Gram + and - wide spectrum
Mode of Action: Single Site	Mode of Action: Multiple Sites
Resistance: Highly potent resistance	Resistance: Impossible resistance
Action on Biofilm: NIL	Action on Biofilm: Removes Biofilm due to biosurfactants
Action on Fungi: NIL	Action on Fungi: Highly Fungicidal through Fengycin and Fusaricin
Flora: will kill it	Flora: Substitute with better flora
Digestion: NIL	Digestion: Production of 15 complex of enzymes
13 days to be free out the chicken body	Decomposition in less than 3 hours from chicken body

Materials & Methods

In-Vitro Analysis

Novo Biotic® Multi-strains FD 777 has the capability of producing 34 types of Lipopeptides which has a wide spectrum of killing effect on *Salmonella*, *E. Coli*, *Pseudomonas aeruginosa*, *Clostridium*, *Staphylococcus aureus* etc.

Below procedure describes the *in-vitro* killing test of Novo Biotic® against:

a) *Salmonella enterica subsp. enterica serovar Enteritidis* ATCC® 13076

b) mix of three *Escherichia coli* O157:H7 (ATCC 43895, ATCC 43889, ATCC 51658)

c) *Staphylococcus aureus* ED711

d) *Pseudomonas aeruginosa* PA416A

i. 50 microliters of the above bacterial (Pathogen) were incubated overnight at 37°C in 20ml of Tryptic Soy Broth at 200rpm shaker.

ii. 100 microliters of the suspension were spread onto TSA plates, Trypticase Soy Broth with 1.5% Agar except for the staphylococcus which contains 0.25% Agar.

- iii. 5 microliters of Novo Biotic ® (1/1000 dilution) were deposited on a disk on lawns of the above pathogens.
- iv. Plates were incubated at 25°C for 48 hours.
- v. The inhibition zones (Clear haloes) were measured in mm as below Figure 1. guideline.

Figure 1: Novo Biotic ® killing efficacy guideline.

In-Vivo Analysis

Birds Husbandry

One-Day-Old Ross 308 AP as hatched broiler chicks were delivered to the farm located in south of Lebanon during December 2019. All farms are semi-open system. The environmental conditions including temperature, humidity and ventilation of the farm were maintained and regulated according to the Aviagen breeder guidelines, by an automatic climate controller during the

trial.

Feed Dietary and Treatment

A 3 phases feeding program was followed: starter (fed from day 0 to 14), grower (fed from 14 to 28), finisher (fed from 28 to 35).

The nutrient value of the feed given by the supplier is shown in Table 2.

Table 2: calculated nutrient composition (%) of the feed.

Nutrients	Unit	Level		
		Starter	Grower	Finisher
Crude Protein	%	23.00	22.00	21.00
ME Energy	Kcal/kg	3150	3200	3250
Linoleic acid	%	1.50	1.30	1.20
Lysine	%	1.25	1.18	1.12
Methionine	%	0.50	0.48	0.45
M+C	%	0.90	0.88	0.85
Threonine	%	0.85	0.83	0.75
Tryptophan	%	0.24	0.22	0.20
Calcium	%	0.95	0.90	0.85
Phosphorus	%	0.50	0.45	0.42
Sodium	%	0.16	0.16	0.16
Chloride	%	0.20	0.18	0.15
Potassium	%	0.55	0.55	0.55

ME stands for Metabolizable Energy.

Experimental Design

Trial was conducted as per below design:

a) Block 1: 9500 Birds considered as control whereas Avilamycin is added 150g per DMton of feed. No Novo Biotic ® is added to drinking water.

b) Block 2: Replica of Block 1 with 9500 Birds. whereas Avilamycin is added 150g per DMton of feed. No Novo Biotic ® is added to drinking water.

c) Block 3: 8900 Birds whereas feed still containing Avilamycin 150g per DMton and added Novo Biotic ® to the drinking water at 1L per 1000L first 7 days and later 1L per 1000L of drinking water 4 days a week till DOD.

d) Block 4: Replica of Block 3 with 8160 Birds whereas

feed still containing Avilamycin 150g per DMton and added Novo Biotic ® to the drinking water at 1 L per 1000 L first 7 days and later added 1 L per 1000 L of drinking water 4 days a week till DOD.

e) Block 5: 8900 Birds whereas Avilamycin stopped from the feed and only added Novo Biotic ® to the drinking water at 1L per 1000L first 7 days and later added 1L per 1000L of drinking water 4 days a week till DOD.

f) Block 6: Replica of Block 5 with 8160 Birds whereas Avilamycin stopped from the feed and only added Novo Biotic ® to the drinking water at 1L per 1000L first 7 days and later added 1L per 1000L of drinking water 4 days a week till DOD.

Table 3 clearly summarize the combination:

Table 3: Trial Design.

Block/Group Number	Building Number	Birds	Avilamycin	Novo Biotic ®
1	1	9500	150 g	0L
2	1	9500	150 g	0L
3	2	8850	150 g	1L
4	3	8850	150 g	1L
5	2	8160	0 g	1L
6	3	8160	0g	1L

Vaccination Program

Vaccination was performed as per below table (Table 4).

Table 4: Vaccination Program.

Age	Vaccination	Route
Day 1	ND	Spraying
Day 1	IB	Spraying
Day 1	ND	Drinking Water
Day 1	AI	Injection
Day 7	IBD	Drinking Water
Day 15	IBD	Drinking Water
Day 22	IBD + ND	Spraying

Broiler Performance

Body Weight (BW) and Feed Intake (FI) were recorded weekly (day 0, 7, 14, 21, 28, 35 and at DOD) as an average per Block. The feed conversion ratio (FCR) is the amount of feed ingested by an animal which can be converted into one kilo of live weight. The economic FCR is calculated as per below formula:

$$\frac{\text{Total amount of feed ingested}}{\text{Body Weight}}$$

Body Weight is the weight of animals that can be accepted at the slaughterhouse. Feed conversion ratio (FCR) adjusted for mortalities was calculated for each Block. Mortality rate per treatment was calculated for the entire trial period. All results are calculated based on the average between block 1 and 2, block 3 & 4 and block 5 & 6. European Production Efficiency Factor also was calculated as per below formula:

$$\frac{\text{Average Weight} \times \% \text{ livability}}{\text{FCR} \times \text{Day of Depletion (DOD)}} \times 100$$

Serological Analysis

5 Blood samples were taken from each group of blocks. With three groups total will be 15 samples per reading. Samples were tested (titers) for IBD (Infectious bursal disease), IBV (avian infectious bronchitis virus), NDV (Newcastle disease virus), AI (Avian Influenza) and MG/MS (Mycoplasma). Titers noted for days 0, Days 20 and Days 40.

Statistical Analysis

Statistical analysis was performed using online statistical tools. The significance between treatments was determined by an analysis of variance with the general linear model. In all cases the level of statistical significance was $P < 0.05$ (Table 5).

Table 5: Inhibition zones on all pathogens.

Pathogens	Diameter (mm) of growth inhibition zone (including well diameter)*
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Enteritidis</i> ATCC® 13076	30.00 +/- 0.5
mix of three <i>Escherichia coli</i> O157:H7 (ATCC 43895, ATCC 43889, ATCC 51658)	31.00 +/- 0.41
<i>Staphylococcus aureus</i> ED711	25.00 +/- 0.29
<i>Pseudomonas aeruginosa</i> PA416A	26.00 +/- 0.41

Screening for Extracellular Enzymatic Activity of Novo Biotic®

In order to quantify the International Units of the enzyme, thermofisher test kits were used.

Results & Discussion

In-Vitro Analysis

Below figures describe the test results along with the inhibition zones. (Figures 2-4)

Figure 2: in vitro efficiency of Novo Biotic® against *Salmonella enterica* subsp. ATCC® 13076.

Figure 3: in vitro efficiency of Novo Biotic® against mix of three *Escherichia coli* O157:H7 (ATCC 43895, ATCC 43889, ATCC 51658).

Figure 4: In vitro efficiency of Novo Biotic® against *Staphylococcus aureus* ED711.

Broiler Performance

Table 6: Average Broiler Performance Results.

	Control Only AGP Group 1	AGP With Novo Group 2	Novo without AGP Group 3
Body Weight (g)			
Day 0	40g	37g	37g
Day 7	176g	164g	159g
Day 14	431g	416g	429g
Day 21	903g	911g	908g
Day 28	1,430g	1,465g	1,453g
Day 35	2,129g	2,185g	2,097g
DOD*	2,605g	2,600g	2,410g
*Day of Depletion DOD Group 1 & 2 at 40 Days			
*Day of Depletion DOD Group 3 at 39 Days			
Feed Intake g/bird			
0 to 7 days	127g	141g	137g
0 to 14 days	483g	457g	513g
0 to 21 days	1,050g	966g	1,020g
0 to 28 days	1,866g	1,660g	1,815g
0 to 35 days	3,363g	3,233g	3,103g
DOD*	4,351g	4,044g	3,698g
FCR			
35 Days	1.58	1.48	1.48
DOD*	1.67	1.56	1.53
Mortality			
35 Days	3.57%	4.50%	4.21%
DOD*	4.25%	6.30%	5.02%
EPF			
DOD*	371	398	389

Table 6 shows all results calculated based on the average between block Group 1 (mean of Blocks 1 and 2), Group 2 (mean of Blocks 3 & 4) and Group 3 (mean of Blocks 5 & 6).

Serological Analysis

Tables 7-9 reveal all serological analysis at day old chicks, 20 days and 40 days, prior depletion.

Table 7: Serological Analysis Day Old Chicks.

	Titers Day 1 Broiler					
Flocks	N	IBD	IBV	NDV	AI	Mg/Ms
Control	1	5527	2857	2063	573	497
	2	4992	2348	2011	857	760
	3	4992	2493	2020	389	328
	4	4903	2276	1838	685	601
	5	6066	3078	1394	610	531
Only Novo Biotic	6	4726	2493	1786	867	769
	7	5886	2931	2503	471	403
	8	5796	3374	1631	685	601
	9	5259	2450	1717	1229	328
	10	5643	3004	2414	1140	395
Novo Biotic + AGP	11	5393	2529	2151	781	689
	12	5616	2850	1976	686	602
	13	5321	2909	1933	920	818
	14	4948	2784	2063	944	840
	15	5652	2385	2832	906	804

Table 8: Serological Analysis 20 Days Old Chicks.

	Titers Day 20 Broiler					
Flocks	N	IBD	IBV	NDV	AI	Mg/Ms
Control	1	9096	6435	6871	573	497
	2	7983	5733	8425	667	583
	3	7798	6122	8230	344	286
	4	8075	6044	8523	676	592
	5	8631	6592	9113	993	885
Only Novo Biotic	6	7614	6435	8034	867	769
	7	7431	6278	7840	462	395
	8	8603	7278	9083	676	592
	9	8260	6985	7626	1249	328
	10	7881	6662	6679	657	575
Novo Biotic + AGP	11	7752	6552	8181	667	583
	12	7679	6490	7161	867	769
	13	7614	6435	8034	1140	1021
	14	8075	6827	8523	993	885
	15	7891	6670	7257	877	778

Table 9: Serological Analysis 40 Days Old Chicks.

Flocks	Titers Day 1 Broiler					
	N	IBD	IBV	NDV	AI	Mg/Ms
Control	1	2067	1708	2151	480	412
	2	2150	1778	2238	573	497
	3	2233	1849	2238	389	328
	4	1100	885	1126	667	583
	5	1903	1568	1976	582	506
Only Novo Biotic	6	1577	1291	1631	867	328
	7	1497	1222	1126	462	395
	8	1658	1360	1717	676	592
	9	2233	1849	2326	1249	328
	10	2399	1990	2503	1150	412
Novo Biotic + AGP	11	1337	1086	1377	867	769
	12	1497	1222	1546	685	601
	13	1456	1188	1504	954	849
	14	1658	1360	1717	906	804
	15	1985	1637	2063	877	778

Anova Results as below table

Table 10 summarizes the Anova analysis of the serological results performed on day 1 old chicks between group for every disease. Majority of the analysis showed no significant difference at $P < 0.05$ except for the:

a) Avian Influenza between the control group treated only

with AGP and the group treated with AGP and Novo Biotic at $0.03 < 0.05$

b) Mg/Ms titers between: firstly, the Groups the control treated with AGP and the one treated with AGP and Novo Biotic and secondly between the group treated with only Novo Biotic and the group treated with Novo Biotic and AGP at $0.02 < 0.05$

Table 10: Anova results.

P-Value at Day 1 chicks				
		Control	Only Novo	Novo + AGP
IBD	Control	1.00	0.60	0.73
	Only Novo	0.60	1.00	0.76
	Novo + AGP	0.73	0.76	1.00
IBV	Control	1.00	0.32	0.67
	Only Novo	0.32	1.00	0.44
	Novo + AGP	0.67	0.44	1.00
NDV	Control	1.00	0.53	0.15
	Only Novo	0.53	1.00	0.48
	Novo + AGP	0.15	0.48	1.00
AI	Control	1.00	0.14	0.03
	Only Novo	0.14	1.00	0.84
	Novo + AGP	0.03	0.84	1.00
Mg/Ms	Control	1.00	0.69	0.03
	Only Novo	0.69	1.00	0.02
	Novo + AGP	0.03	0.02	1.00

In order to better interpret the results below charts reveals the titers of the 3 groups for every disease along with the disease evaluation (Figures 5-9). All flocks were being protected and

uninfected for all the diseases as all titers are below the infection baseline.

Figure 5: IBD Titer Day 1 Broiler.

Figure 6: IBV Titer Day 1 Broiler.

Figure 7: NDV Titer Day 1 Broiler.

Figure 8: AI Titer Day 1 Broiler.

Figure 9: Mg/Ms Titer Day 1 Broiler.

Anova Results as below table

Table 11 summarizes the Anova analysis of the serological results performed on day 20 old chicks between group for every disease.

Table 11: Anova results.

P-Value at Day 20 chicken				
		Control	Only Novo	Novo + AGP
IBD	Control	1.00	0.29	0.07
	Only Novo	0.29	1.00	0.51
	Novo + AGP	0.07	0.51	1.00
IBV	Control	1.00	0.051	0.03
	Only Novo	0.051	1.00	0.51
	Novo + AGP	0.03	0.51	1.00
NDV	Control	1.00	0.49	0.40
	Only Novo	0.49	1.00	0.96
	Novo + AGP	0.40	0.96	1.00
AI	Control	1.00	0.45	0.08
	Only Novo	0.45	1.00	0.43
	Novo + AGP	0.08	0.43	1.00
Mg/Ms	Control	1.00	0.77	0.08
	Only Novo	0.77	1.00	0.03
	Novo + AGP	0.08	0.03	1.00

Majority of the analysis showed no significant difference at $P < 0.05$ except for the:

c) IBV between the control group treated only with AGP and the group treated with AGP and Novo Biotic at $0.03 < 0.05$

d) Mg/Ms titers between the group treated with Novo Biotic alone and the group treated with Novo Biotic and AGP at

In order to better interpret the results below charts reveals the titers of the 3 groups for every disease along with the disease evaluation (Figures 10-14). All flocks were being protected and uninfected for all the diseases as all titers are below the infection baseline.

Figure 10: IBD Titer Day 20 Broiler.

Figure 11: IBV Titer Day 20 Broiler.

Figure 12: NDV Titer Day 20 Broiler.

Figure 13: AI Titer Day 20 Broiler.

Figure 14: Mg/Ms Titer Day 20 Broiler.

Table 12 summarizes the Anova analysis of the serological results performed on day 40 old chicks between group for every disease. Majority of the analysis showed no significant difference at $P < 0.05$ except for the:

e) NDV between the control group treated with AGP and Novo Biotic at $0.02 < 0.05$

f) AI titers between the group control and the group treated with Novo Biotic + AGP at $0.00 < 0.05$

g) Mg/Ms titers between the control group treated only with AGP and the group treated with AGP and Novo Biotic at $0.00 < 0.05$ & between the group treated with Novo Biotic alone and the group treated with Novo Biotic + AGP at $0.00 < 0.05$

Table 12: Anova results.

P-Value at Day 40 chicken				
		Control	Only Novo	Novo + AGP
IBD	Control	1.00	0.95	0.22
	Only Novo	0.95	1.00	0.22
	Novo + AGP	0.22	0.22	1.00
IBV	Control	1.00	0.94	0.22
	Only Novo	0.94	1.00	0.22
	Novo + AGP	0.22	0.22	1.00
NDV	Control	1.00	0.80	0.02
	Only Novo	0.80	1.00	0.06
	Novo + AGP	0.02	0.06	1.00
AI	Control	1.00	0.05	0.00
	Only Novo	0.05	1.00	0.88
	Novo + AGP	0.00	0.88	1.00
Mg/Ms	Control	1.00	0.42	0.00
	Only Novo	0.42	1.00	0.00
	Novo + AGP	0.00	0.00	1.00

In order to better interpret the results below charts reveals the titers of the 3 groups for every disease along with the disease evaluation (Figures 15-19). All flocks were being protected and uninfected for all the diseases as all titers are below the infection baseline. Although Anova results revealed some significant differences but all above serological titers show protection in some cases but uninfected flocks in all cases (Table 13).

Screening for Extracellular Enzymatic Activity of Novo Biotic®

The bacterial strains of the Bacillus FD777 isolates, after 10 minutes of adding in the drinking water, presented extracellular enzymatic activity as below:

Table 13: Extracellular Enzymes Quantification.

Enzymes	I.U Per 1L of Drinking Water
Lipase	42
Protease	46
Amylase	31
Cellulase	23
Xylanase	26
Chitinase	29
Alginate Lyase	28

Figure 15: IBD Titer Day 40 Broiler.

Figure 16: IBV Titer Day 40 Broiler.

Figure 17: NDV Titer Day 40 Broiler.

Figure 18: AI Titer Day 40 Broiler.

Figure 19: Mg/Ms Titer Day 40 Broiler.

Conclusion

a) When adding Novo Biotic ® to AGP and comparing group 1 and 2 we can clearly see that FCR of group 2 (1.56) is way better than FCR of Group 1 (1.67). Novo Biotic ® promoted the development of positive bacterial flora in the digestive system of the chicken. The FD 777 Strains populations in the GIT are associated with enhanced animal performance, reflecting more efficient digestion and improved immunity. The FD 777 increased the digestion and absorption of nutrients essentially the amino acids due to high production of Protease also specially the amylase which also increase the body weight gain and improvement in feed use efficiency. Previous studies and analysis on Novo Biotic ® Studies show a complex of synergy between the activity of 15 complexes enzymes to include: Protease, metalloproteases, Amylase, Lipase, Esterase, urease, Xylanase, Cellulase and Phytase.

b) By removing the Avilamycin AGP and keeping Novo Biotic ®, we can clearly notice a better drop in the FCR between Group 2 (1.56) and Group 3 (1.52) and this is due the effect of the AGP on the Novo Biotic ® performance. Accordingly, always stop AGP from feed while using Novo Biotic ® to have the optimum performance of the FD 777 strains. Dropping the FCR from 1.67 to 1.53 and stopping the AGP has a substantial ROI (Return on Investment) to the client.

c) At 1 L per 1000 L of water as per above program, Novo Biotic ® completely replaces the Avilamycin AGP with better FCR (approx 14 points) accordingly the farmer can claim Antibiotic free chicken.

d) Due to 34 + Lipopeptides, Novo Biotic ® has the ability to complete inhibit and kill the pathogens such as *E. Coli*, *Salmonella*, *Pseudomonas* and *Staphylococcus* with a minimum inhibition zone of 25 mm.

References

- Visek WJ (1978) The Mode of Growth Promotion by Antibiotics. Journal of Animal Science 46: 1447-1469.
- Bartlett JG, Gilbert DN, Spellberg B (2013) Seven ways to preserve the miracle of antibiotics. Clin Infect Dis 56(10): 1445-1450.
- Centers for Disease Control and Prevention, Office of Infectious Disease (2013) Antibiotic resistance threats in the United States.
- Congressional Research Service Report (2005) Life expectancy in the United States. March.
- Golkar Z, Bagazra O, Pace DG (2014) Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. J Infect Dev Ctries 8(2): 129-136.
- Gross M (2013) Antibiotics in crisis. Curr Biol 23(24): R1063-R1065.
- Blackall PJ (1988) Antimicrobial drug resistance and the occurrence of plasmids in Haemophilus paragallinarum. Avian Dis 32(4): 742-747.
- Luna Galaz GA, Morales Erasto V, Peñuelas Rivas CG, Blackall PJ, Soriano-Vargas E (2016) Antimicrobial sensitivity of Avibacterium paragallinarum isolates from four Latin American countries. Avian Dis 60: 673-676.
- Van TT, Nguyen HN, Smooker PM, Coloe PJ (2012) The antibiotic resistance characteristics of non-typhoidal Salmonella enterica isolated from food-producing animals, retail meat and humans in South East Asia. Int J Food Microbiol 154(3): 98-106.
- Simoneit C, Burow E, Tenhagen BA, Käsbohrer A (2015) Oral administration of anti-microbials increase antimicrobial resistance in E. coli from chicken-a systematic review. Prev Vet Med 118(1): 1-7.
- Tadesse DA, Zhao S, Tong E, Ayers S, Singh A, et al. (2012) Antimicrobial drug resistance in Escherichia coli from humans and food animals, United States 1950-2002. Emerg Infect Dis 18(5): 741-749.
- Costa MC, Bessegatto JA, Alfieri AA, Weese JS, Filho JAB, et al. (2017) Different antibiotic growth promoters induce specific changes in the cecal microbiota membership of broiler chicken. PLoS ONE 12(2): e0171642.
- Fasina YO, Newman MM, Stough JM, Liles MR (2015) Effect of Clostridium perfringens infection and antibiotic administration on microbiota in the small intestine of broiler chickens. Poult Sci.
- Lushniak BD (2014) Antibiotic resistance: a public health crisis. Public Health Rep 129(4): 314-316.
- Michael CA, Dominey Howes D, Labbate M (2014) The antibiotic resistance crisis: causes, consequences, and management. Front Public Health 2:145.
- Piddock LJ (2012) The crisis of no new antibiotics - what is the way forward? Lancet Infect Dis 12(3): 249-253.
- Read AF, Woods RJ (2014) Antibiotic resistance management. Evol Med Public Health 1:147.
- Sengupta S, Chattopadhyay MK, Grossart HP (2013) The multifaceted roles of antibiotics and antibiotic resistance in nature. Front Microbiol 4: 47.
- Spellberg B, Gilbert DN (2014) The future of antibiotics and resistance: a tribute to a career of leadership by John Bartlett. Clin Infect Dis 59 suppl 2: S71-S75.
- (2013) The antibiotic alarm. Nature 495(7440): 141.
- Viswanathan VK (2014) Off-label abuse of antibiotics by bacteria. Gut Microbes 5(1): 3-4.
- World Health Organization (2014) Antimicrobial Resistance: Global Report on Surveillance.
- Marshall BM, Levy SB (2011) Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev 24(4): 718-733.
- Nhung NT, Chansiripornchai N, Carrique Mas JJ (2017) Antimicrobial Resistance in Bacterial Poultry Pathogens: A Review. Front Vet Sci 4: 126.
- Van Boeckel T, Brower C, Gilbert M, Grenfell BT, Levin SA, et al. (2015) Global trends in antimicrobial use in food animals. PNAS.
- Guillot JF, Coudray MC, Chaslus-Dancla ET, Lafont JP (1977) Phenotypes Of Escherichia Coli Of avian origin with respect to aminoglycosides. Med Diseases Infect 7: 449e55.
- Diarra MS, Malouin F (2014) Antibiotics in Canadian poultry productions and anticipated alternatives. Front Microbiol 5: 282.
- Pakpinyo S, Sasipreeyajan J (2007) Molecular characterization and determination of antimicrobial resistance of mycoplasma gallisepticum isolated from chickens. Vet Microbiol 125(1-2): 59-65.
- Manning J, Gole V, Chousalkar K (2015) Screening for salmonella in backyard chickens. Prev Vet Med 120(2): 241-245.

30. Castanon JIR (2007) History of the use of antibiotic as growth promoters in European poultry feeds. *Poult Sci* 86(11): 2466-2471.
31. (2014) *Health Rep* 129(4): 314-316.
32. Zhang ZF, Kim IH (2014) Effects of multi-strain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers. *Poult Sci* 93(2): 364-370.
33. Wright GD (2014) Something new: revisiting natural products in antibiotic drug discovery. *Can J Microbiol* 60(3): 147-154.
34. Silbergeld EK, Graham J, Price LB (2008) Industrial food animal production, antimicrobial resistance, and human health. *Annu Rev Publ Health* 29:151-169.

This work is licensed under Creative Commons Attribution 4.0 License
DOI: [10.19080/TTSR.2021.04.555648](https://doi.org/10.19080/TTSR.2021.04.555648)

**Your next submission with Juniper Publishers
will reach you the below assets**

- Quality Editorial service
- Swift Peer Review
- Reprints availability
- E-prints Service
- Manuscript Podcast for convenient understanding
- Global attainment for your research
- Manuscript accessibility in different formats
(Pdf, E-pub, Full Text, Audio)
- Unceasing customer service

Track the below URL for one-step submission
<https://juniperpublishers.com/online-submission.php>