

Naturalguard as essential oil blend supplement to reduce the disease risk and increase the productivity of Poultry and Cattle

Rhea Natural Sciences

CHARACTERISTICS OF NATURALGUARD



NATURALGUARD DILUTION METHOD

Mix recommended dose of Naturalguard in 10 liter of clean water.

Mix well for 2 to 3 minutes.

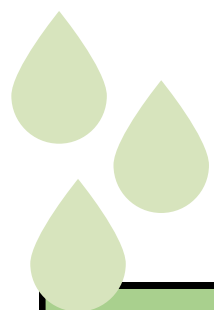
Mix Naturalguard solution in the drinking water tank



POULTRY



RECOMMENDED DOSE FOR CHICKEN



Application Method		Dose	Frequency
Mix in the feed	Preventive	2 kg per ton feed	Daily
	Treatment	4 kg per ton feed	Daily
Mix in the drinking water	Preventive	20 ppm	Daily
	Treatment	40 ppm	For 7-10 consecutive days

Summary of Laboratory level trials and field observation

Beneficial Properties	Test Methodology	Results
Naturalguard- salt as Antibiotics and Growth Promoter replacement in chicken feed	<i>In vivo</i> at IPB Bogor and CPI Jakarta	Growth performance and survival rate increased in Poultry. Obtained higher IP value.
Naturalguard –liquid as Antibiotics and Growth Promoter replacement in chicken	<i>In vivo</i> at IPB Bogor and CPI Jakarta	Growth performance and survival rate increased in Poultry. Obtained higher IP value.
Naturalguard – enhance the meat quality of Broiler chicken	<i>In vivo</i> at IPB Bogor and CPI Jakarta	Enhanced the meat quality and texture with higher SR and overall IP value.
Naturalguard – enhance the quality of Egg	<i>In vivo</i> at UGM Yogyakarta	Enhanced the quality of eggs, lowers cholesterol and Uric acid level
Naturalguard against highly Pathogenic Avian Influenza (HPAI H5N1)	<i>In vivo</i> at IPB Bogor	Effective, 100 % effective in preventing H5N1 transmission
Naturalguard against Highly Pathogenic Newcastle Disease (ND)	In vivo at CPI, Jakarta	Effective, 100 % effective in preventing ND transmission
Naturalguard-Deactivation of Avian Infectious Bronchitis Virus H120	In Vitro Padjajaran Univ & BPA, Indonesia	Effective, 100 % effective in preventing ND transmission up to certain dilution
Naturalguard – enhance the meat quality of Beef Cattle	<i>In vivo</i> at UGM Yogyakarta	The quality of meat increased with increase in total weight gain (0.17 kg)
Naturalguard – reduces the transportation stress in Cattle	<i>In vivo</i> at UGM Yogyakarta	Reduced the transportation stress and faster recovery period
Naturalguard – enhance the milk quality of cow	<i>In vivo</i> at UGM Yogyakarta	Growth performance and milk production increased up to 22 %.

Antibiotics and Growth promoter replacement

Serial No.	Performance Parameters	Naturalguard – Salt (In feed)	Control
1.	No. of chicken	1400	1400
2.	Feed consumption (g/Bird)	70.55± 3.33 ^a	68.80±6.00 ^a
3.	Water consumption (mL/Bird)	224.27±10.80 ^a	190.01±16.72 ^b
4.	Mortality percentage	5.62	10
5.	FCR	1.59±0.07 ^a	1.65±0.14 ^a
6.	Survival Rate (%)	94.38	90.0
7.	Final Population (No.)	151	144
8.	Total Final Biomass (kg)	235	210
9.	Total Feed consumption (kg)	373	347
10.	Performance Index (IP) value	271	233

- The *in vivo* trial was established to determine the role of Naturalguard as antibiotics and growth promoter replacement in the broiler chicken feed.
- The obtained results showed that chicken on Naturalguard performed better with increased weight gain, lower FCR, higher survival rate, and better IP values.
- The performance of Naturalguard was equally good as consumed in feed and in the drinking water.
- The obtained results showed that Naturalguard is a potential candidate to replace AGP in chicken feed.
- Application of Naturalguard (2 kg / ton feed) and Naturalguard Liquid (0.5 mL / L drinking water) were proven safe (not toxic to chickens) based on liver function (SGPT and SGOT), kidney function (urea and creatinine), and description of PA and HP from the liver and kidney.
- The cost/benefit analysis shows that the application of NG and NG-L increases the profitability by 50% and 37% respectively.

Antibiotics and Growth promoter replacement

Serial No.	Performance Parameters	Naturalguard – Liquid (In drinking)	Control
1.	No. of chicken	1400	1400
2.	Feed consumption (g/Bird)	71.84±3.23 ^a	68.80±6.00 ^a
3.	Water consumption (mL/Bird)	186.32±8.26 ^a	190.01±16.72 ^b
4.	Mortality percentage	8.75	10
5.	FCR	1.60±0.07 ^a	1.65±0.14 ^a
6.	Survival Rate (%)	91.25	90.0
7.	Final Population (No.)	146	144
8.	Total Final Biomass (kg)	229	210
9.	Total Feed consumption (kg)	367	347
10.	Performance Index (IP) value	262	233

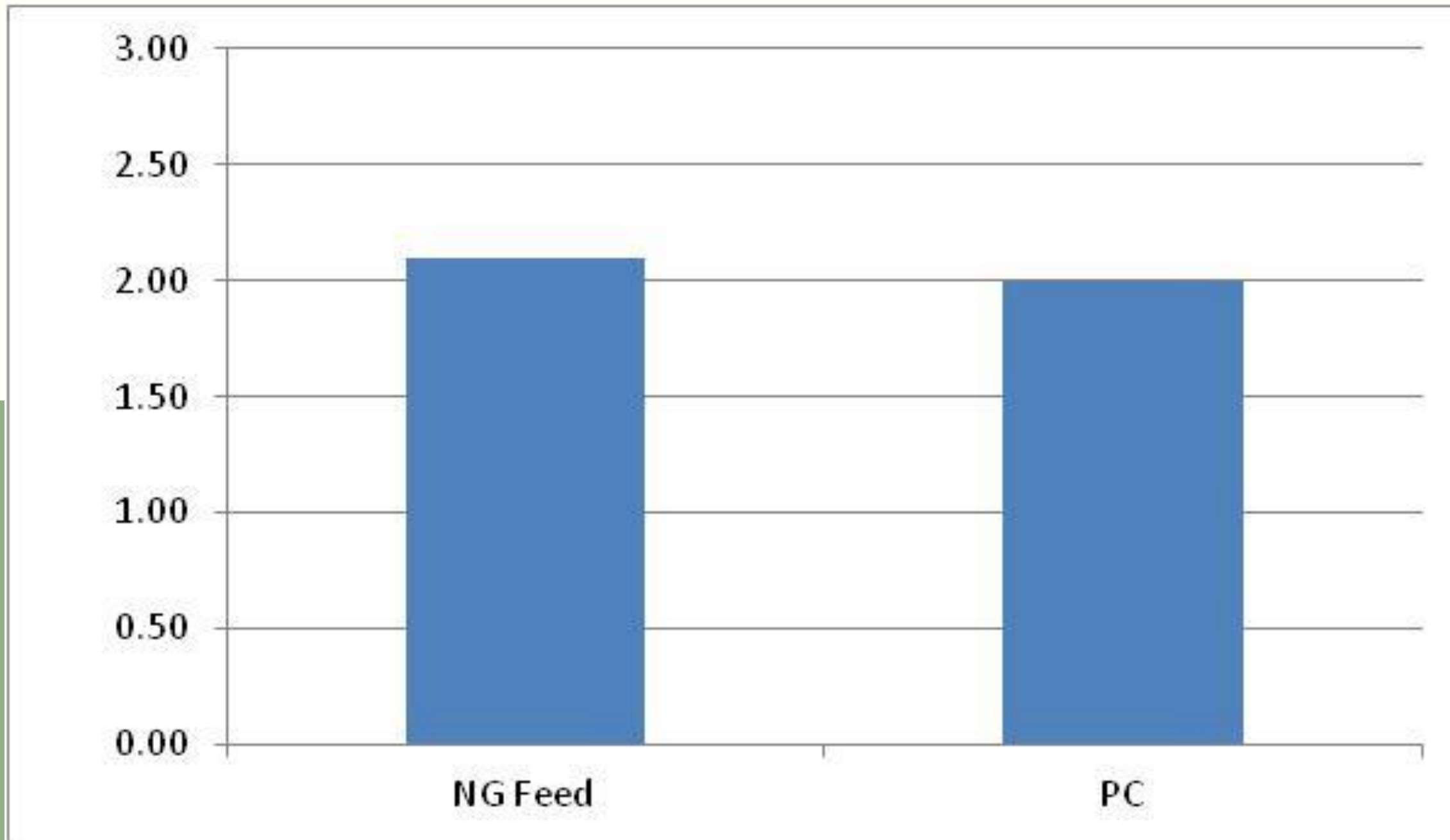
- The *in vivo* trial was established to determine the role of Naturalguard as antibiotics and growth promoter replacement in the broiler chicken feed.
- Naturalguard group had better survival rate, better FCR and higher biomass.
- The IP value of the NG group was better compared to the control.
- The obtained data showed that Naturalguard is capable to replace antibiotics and growth promoters in the feed.
- The cost/benefit analysis shows that the application of NG and NG-L increases the profitability by 50% and 37% respectively.

Antibiotics and Growth promoter replacement

Serial No.	Performance Parameters	Naturalguard – Salt (In feed)	Control
1.	No. of chicken (COBB 500)	400	400
2.	Body weight (g)	1935 ±82.44	1894 ±81.39
3.	ADG (g/day)	49.25 ±2.28	49.25 ±3.82
4.	Feed intake (g)	3032 ±90.32	2894 ±91.60
5.	Water intake (mL/Bird)	6843 ±180.45	6780 ±198.33
7.	FCR	1.591 ±0.03	1.579 ±0.06
8.	EEF (Performance Index (IP) value)	380 ±23.27	375.59 ±25.04
9.	Cumulative Mortality (%)	8.58	10.52

- The *in vivo* trial was established to determine the role of Naturalguard as antibiotics and growth promoter replacement in the broiler chicken feed.
- Naturalguard group had better survival rate, better FCR and higher biomass.
- The IP value of NG group was better compared to the control.
- The obtained data showed that Naturalguard is capable to replace antibiotics and growth promoters in the feed.
- The cost/benefit analysis shows that the application of NG and NG-L increases the profitability by 50% and 37% respectively.

Antibiotics and Growth promoter replacement



- The *in vivo* trial was established to determine the role of Naturalguard as antibiotics and growth promoter replacement in the broiler chicken feed.

- The obtained results showed that Naturalguard was able to enhance the ND-HI titre (log 2 between 2-7) was higher than control birds

NG-Salt Trials conducted in a Research farm, IPB, Jakarta

Meat Quality

Parameter	Control	T 1	T 2
Breast			
moisture(%)	75.32 ± 1.11 ^a	74.70 ± 0.52 ^a	75.13 ± 1.27 ^a
Crude Protein (%)	18.40 ± 1.15 ^a	19.95 ± 0.43 ^a	19.70 ± 0.98 ^a
fat(%)	0.43 ± 0.18 ^a	0.60 ± 0.24 ^a	0.79 ± 0.21 ^a
Carbohydrate (%)	4.29 ± 0.80 ^a	3.38 ± 0.62 ^a	3.09 ± 0.29 ^a
Ash (%)	1.57 ± 0.11 ^a	1.37 ± 0.13 ^{ab}	1.29 ± 0.10 ^b
Thigh			
moisture(%)	75.64 ± 1.58 ^a	76.87 ± 1.11 ^a	76.53 ± 1.22 ^a
Crude Protein (%)	16.15 ± 0.07 ^a	15.32 ± 0.79 ^a	16.47 ± 0.52 ^a
fat(%)	2.20 ± 0.29 ^a	2.57 ± 1.35 ^a	2.07 ± 0.77 ^a
Carbohydrate (%)	4.79 ± 1.33 ^a	4.14 ± 2.23 ^a	3.79 ± 0.96 ^a
Ash (%)	1.23 ± 0.02 ^a	1.10 ± 0.03 ^b	1.12 ± 0.02 ^b
Wing			
moisture(%)	72.83 ± 0.55 ^a	73.92 ± 0.21 ^a	73.79 ± 0.26 ^a
Crude Protein (%)	18.96 ± 0.92 ^a	17.71 ± 0.83 ^a	18.22 ± 1.47 ^a
fat(%)	1.47 ± 0.37 ^a	2.56 ± 0.99 ^a	1.71 ± 0.16 ^a
Carbohydrate (%)	5.51 ± 0.90 ^a	4.48 ± 1.54 ^a	5.15 ± 1.24 ^a
Ash (%)	1.23 ± 0.13 ^a	1.33 ± 0.17 ^a	1.13 ± 0.04 ^a

- The overall meat quality of all groups is almost similar with no significant differences except ash content (%).
- It is considered that the lower the ash content, the better the quality of the meat.

NG-Salt Trials conducted in Research farm, IPB, Jakarta

Blood Parameters

Parameter	Control	T1	T2	Standard
After treatment (H 35)				
Red blood cell ($10^6/\text{mm}^3$)	3.43 ± 0.21^a	3.50 ± 0.22^a	3.48 ± 0.05^a	2.3-3.5
Hematocrit (%)	25.74 ± 1.95^a	26.16 ± 3.01^a	23.64 ± 1.94^a	22-35
Hemoglobin (g%)	11.22 ± 0.27^a	10.80 ± 0.59^a	11.29 ± 0.75^a	7-13
White blood cell ($10^3/\text{mm}^3$)	13.12 ± 3.75^b	15.72 ± 5.17^{ab}	19.40 ± 6.21^a	12-30
Limfosit Lymphocytes (%)	55.17 ± 1.59^b	58.00 ± 3.23^{ab}	61.83 ± 5.95^a	55-66
Monocytes (%)	3.20 ± 1.03^a	2.70 ± 0.67^a	2.90 ± 0.99^a	2-9
Heterophyll (%)	44.40 ± 5.66^a	44.00 ± 7.79^a	38.30 ± 12.20^a	25-30
Eosinophils (%)	0.20 ± 0.426^a	0.60 ± 0.84^a	0.60 ± 0.70^a	0-3
Basophils (%)	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0-3
H/L	0.81 ± 0.11^a	0.76 ± 0.15^a	0.63 ± 0.24^a	0.2-0.8

Parameter	Control	T1 (NG)	T2 (NG-L)	Standard
Trigliserida (mg/dL)	134.50 ± 19.10^a	121.00 ± 49.50^a	113.00 ± 60.80^a	150
Total kolesterol (mg/dL)	149.00 ± 18.40^a	150.50 ± 4.95^a	157.00 ± 39.60^a	125-200
HDL (mg/dL)	93.50 ± 4.24^a	93.50 ± 0.71^a	90.50 ± 27.60^a	min 22
LDL (mg/dL)	31.50 ± 4.95^a	26.50 ± 0.70^a	35.00 ± 2.83^a	max 130
Total protein (g/dL)	2.84 ± 0.59^a	3.13 ± 0.73^a	3.23 ± 0.74^a	2.5-4.5
Albumin (g/dL)	1.00 ± 0.13^a	1.13 ± 0.24^a	1.06 ± 0.16^a	1.6-2.0

Note: different superscripts on the same line showed significantly different results ($p < 0.05$)

- The blood parameters of all the groups are in acceptable range and within the standard level.
- It shows that Naturalguard has No negative impact on the quality of poultry.

NG-Salt Trials conducted in Research farm, IPB, Jakarta

Safety Parameters – Liver and Kidney Function

Parameter	Control	T1 (NG)	T2 (NG-L)	Standard
SGOT (U/L)	242.00±129.90 ^a	237.50±70.00 ^a	241.90±76.20 ^a	70-279
SGPT (U/L)	1.41±0.79 ^a	1.14±0.72 ^a	1.57±0.62 ^b	1-2
Ureum (mg/dL)	1.14±0.36 ^a	1.05±0.70 ^a	0.78±0.57 ^a	0-5
Creatinine (mg/dL)	0.33±0.05 ^a	0.30±0.02 ^a	0.30±0.03 ^a	0.1-0.4

- The safety parameters of all the groups are in acceptable range and within the standard level.
- The tested parameters to determine the function of liver and kidney are in standard range.
- It shows that there is no negative impact observed due to NG and NG-L consumption on chicken during the trial.

Note: different superscripts on the same line showed significantly different results ($p < 0.05$)



NG-Salt Trials conducted at Research farm, UGM, Yogyakarta

Impact on Chicken Egg quality



Paramater	Control (n: 15 Birds)	BAV (N:15 Birds)	SE	P-Value
Egg mass (g)	63.32	63.80	0.638	0.074
Egg Length (cm)	5.72	5.70	0.034	0.481
Egg Width (cm)	4.43	4.42	0.026	0.417
Albumen height (cm)	0.76	0.77	0.021	0.564
Albumen width (cm)	7.40	7.54	0.106	0.105
Yolk height (cm)	1.63	1.67	0.022	0.901
Yolk width (cm)	3.93	3.97	0.038	0.030
Yolk color(cm)	7.66	8.28	0.091	0.439
Shell thick (cm)	0.29	0.30	0.005	0.424
Shell mass (g)	6.53	6.50	0.089	0.305
Haugh Unit	85.50	86.08	1.306	0.519

•The in vivo level trial was established to determine the role of Naturalguard on egg quality improvement in chicken.

•The results showed that chicken on Naturalguard performed better in getting a higher number of eggs per pan by consuming less feed.

•The cholesterol level was lower, and the yolk protein was higher in the chicken which consumed Naturalguard.

•There was an overall improvement in the egg physiochemical quality in the group of chicken which consumed Naturalguard.

Parameters	Control	Natural Guard
Total egg per pan	2.94±0.02 ^a	2.99±0.02 ^b
Feed Intake (g)	119.92±0.19	118.80±0.69
HDA (%)	97.86±0.53 ^a	99.52±0.65 ^b
FCR	1.85±0.02	1.81±0.07

^{a,b} Means in the same row not sharing a common superscript differ significantly at P <0.05.

Parameters	Control	Natural Guard
Cholesterol	12.137±0.76 ^b	9.523±0.47 ^a
Albumen protein ^{ns}	11.300±0.42	11.563±0.17
Yolk protein ^{ns}	17.597±0.46	18.101±0.58
Xanthophyll ^s	0.947±0.05	1.109±0.15

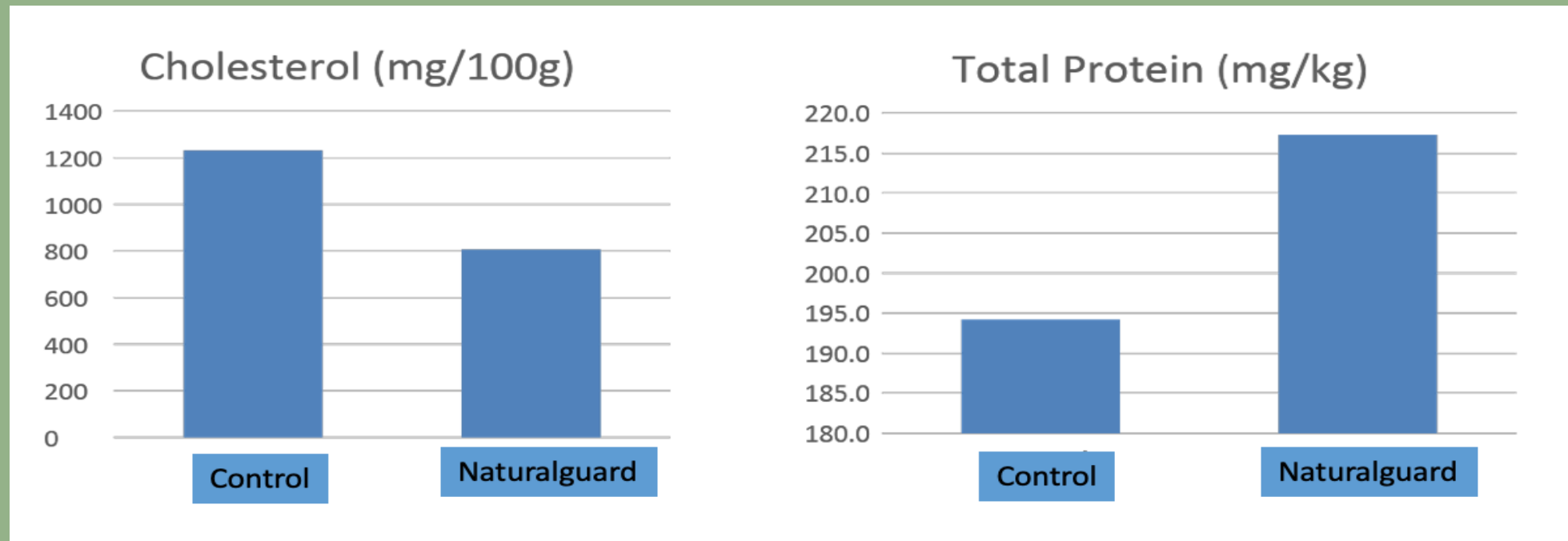
^{a,b} Means in the same row not sharing a common superscript differ significantly at P <0.05

NG-Salt Trials conducted at Research farm, UGM, Yogyakarta

Impact on Chicken Egg quality



Effect of *Naturalguard* on Cholesterol and total protein of egg



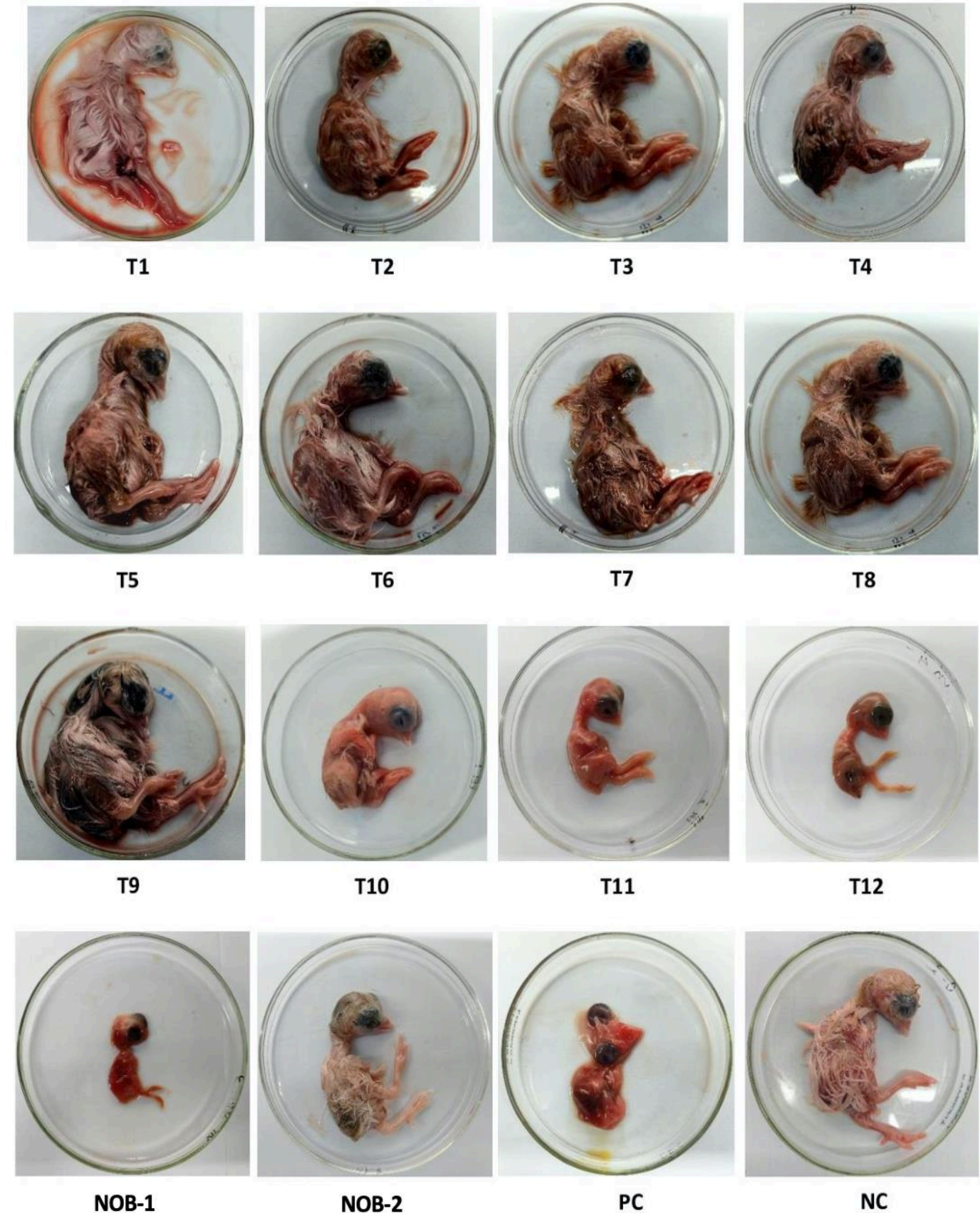
Treatment	Cholesterol (mg/100g)	Protein albumin (mg/kg)
Control	1232.4	194.2
Naturalguard	806.6	217.3

Utilization of *Naturalguard* (2kg/ton feed) had a 34.6% decrease in cholesterol and a 11.9% increase of total protein compared to the control group.

Material / Treatment	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	Naturalguard Control 1	Naturalguard Control 2	Positive Control	Negative Control
Virus/ Allantoid Fluid (mL)	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0
Naturalguard (mL)	2.5	1.25	0.625	0.3125	0.156	0.08	0.04	0.02	0.01	0.005	0.0025	0.00125	5	0.16	0	0
Saline Water (mL)	2.5	3.75	4.375	4.6875	4.998	4.92	4.96	4.98	4.99	4.995	4.9975	4.99875	0	4.84	5	10
Naturalguard (%)	25%	13%	6.3%	3.1%	1.56%	0.8%	0.4%	0.2%	0.1%	0.05%	0.025%	0.0125%	100.0000%	1.6000%	0.0000%	0.0000%

Natural Oil Blend doses against the AIBV Treatment groups (T-1 to T-12), NOB control 1, NOB Control 2, Positive Control, and Negative Control

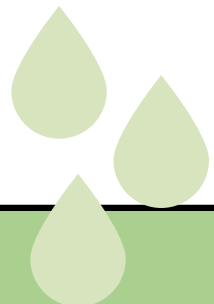
Groups	Total embryos	Embryo Observation		
		Viable Embryos	Dead Embryos	Abnormal /Undeveloped Embryos
T1	5	5	-	-
T2	5	5	-	-
T3	5	5	-	-
T4	5	5	-	-
T5	5	5	-	-
T6	5	5	-	-
T7	5	5	-	1
T8	5	5	-	-
T9	5	5	-	-
T10	5	2	1	2
T11	5	-	2	3
T12	5	-	4	1
NOB Control 1	5	-	5	-
NOB Control 2	5	-	2	3
Positive control	5	2	2	1
Negative control	5	5	-	-



Visual observation of Embryos of different trial groups, Treatment groups (T-1 to T-12), NOB control 1, NOB Control 2, Positive Control, and Negative Control groups.

CATTLE

RECOMMENDED DOSE FOR CATTLE

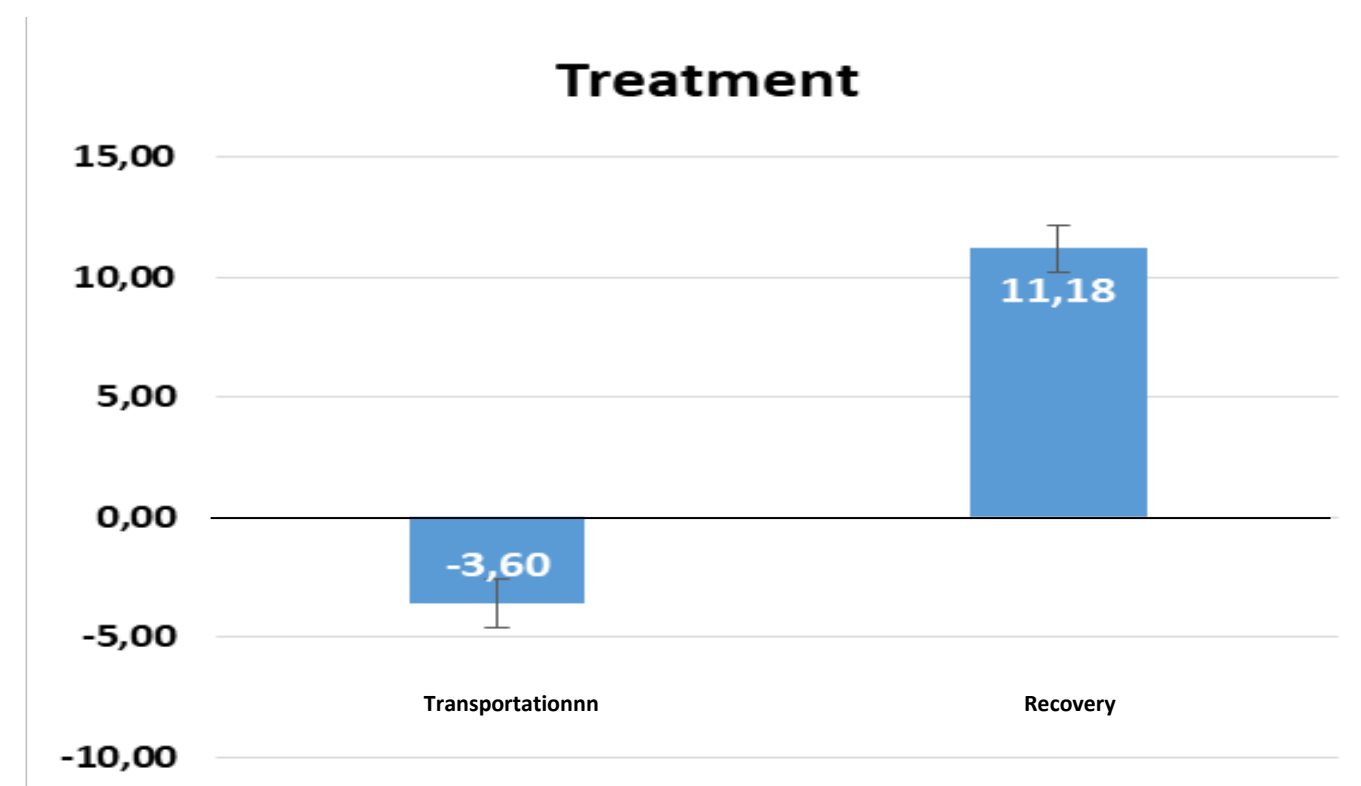
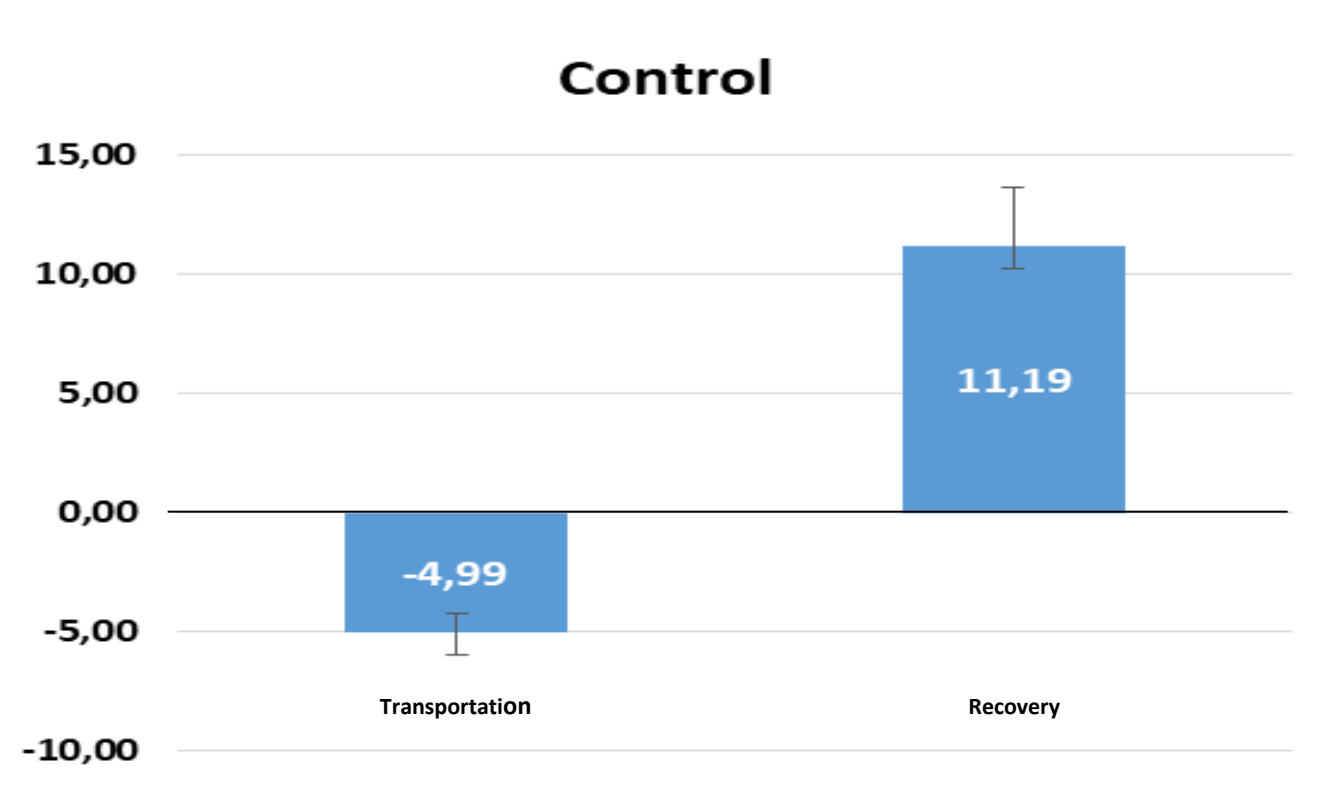


Application Method		Dose	Frequency
Mix in the feed	Preventive	2 kg per ton feed	Daily
	Treatment	4 kg per ton feed	Daily
Mix in the drinking water	Preventive	80 ppm	Daily
	Treatment	160-240 ppm	For 7-10 consecutive days



Item	Treatment		SEM	P-value
	Control	Naturalguard		
Initial BW, kg	87.55	88.35	1.69	0.932
Final BW, kg	128.50	137.30	3.71	0.360
DMI, kg/d	4.42	4.40	0.05	0.146
ADG, kg/d	0.68	0.82	0.03	0.942
G:F	0.156	0.192	0.01	0.168

- An extensive trial was conducted to observe the impact of Naturalguard consumption of stress reduction and recovery periods.
- The results showed that cattle on Naturalguard performed better in getting a higher weight gain and shorter recovery time from stress.
- There was a daily body weight gain of 0.17 kg in the Naturalguard- compared to the control group (0.85 kg vs 0.68 Kg).



Impact on growth and milking capacity

Treatment	Initial body weight	Body weight (60 d)	Gain (kg)
Control	522	556	34
Naturalguard	530	575	45

•An extensive trial was conducted on 56 dairy cattle to observe the impact of Naturalguard consumption on growth and increase in milking capacity.

•The results showed that Cattle on Naturalguard performed better in achieving a higher weight gain and increased milk production (23 % higher).

Parameter	Control (n=28 head)	Naturalguard (n=28 head)
Initial Body weight (kg)	514.20	514.55
Final Body weighy (kg)	547.30	557.35
Total Milk Production (49 d)	551.54	574.15
Average daily production (l)	11.26	11.74
Daily feed intake (kg)	20.39	20.90
Blood plasma metabolism		
Calcium (mg/Kg)	34.56	33.63
Phosphorus (mg/Kg)	107.62	93.70
Magnesium (mg/Kg)	9.49	9.52
Zing (mg/Kg)	0.58	0.40
Iron Fe (mg/Kg)	3.27	3.26
Sodium (mg/Kg)	219.49	216.68
Chloride (mg/Kg)	671.74	662.31

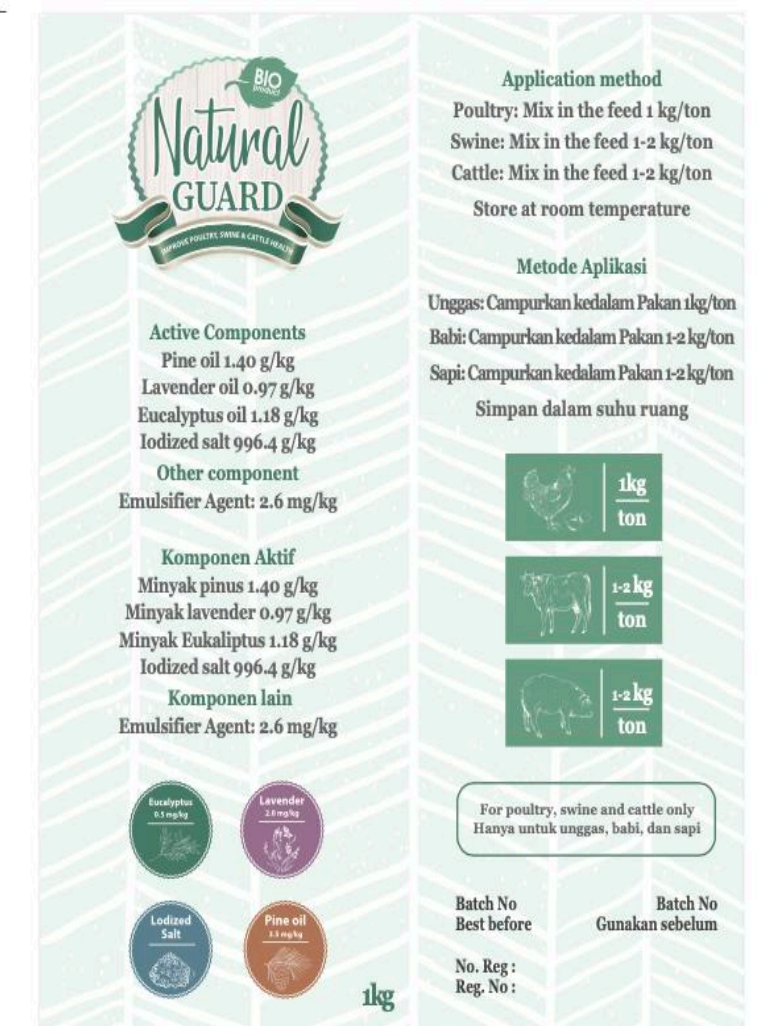
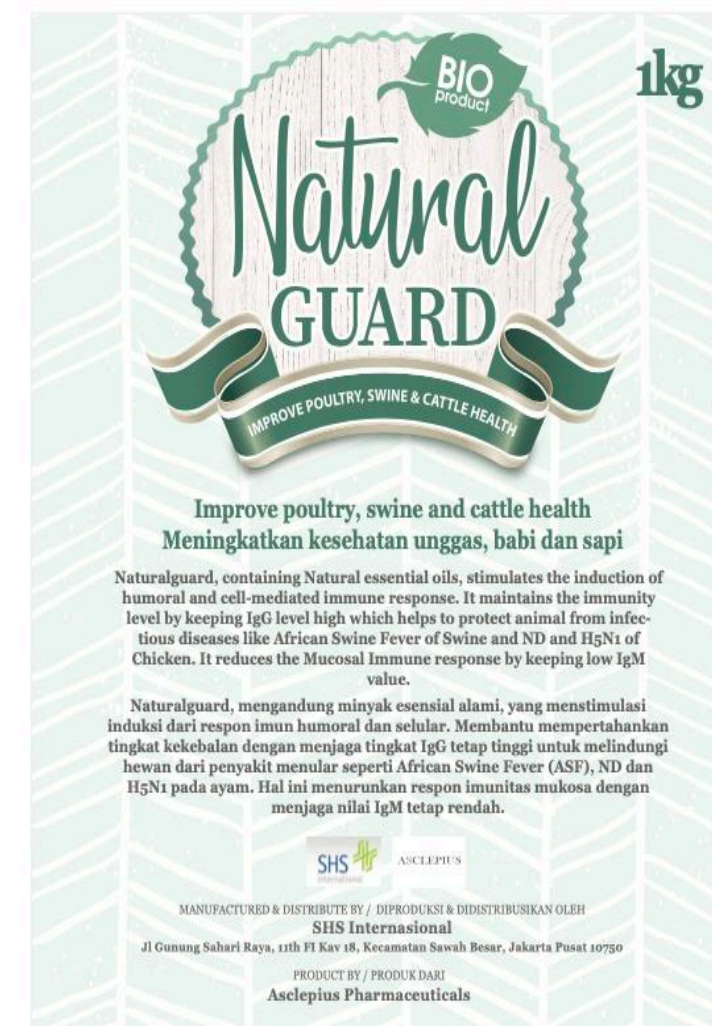
CONCLUSIONS

The developed blend formulation Naturalguard is an effective immunomodulator and anti-pathogenic agent which supports Chicken to grow and lay eggs in its optimum condition, which resulted in better productivity. In Cattle, Naturalguard was shown to promote weight gain and milk production, as well as to reduce stress levels and recovery times during transport.





Salt Pack 1 KG
CMYK 14cm X 17cm



NaturalGuard

We protect your Farm, Naturally



LIQUID 1L



LIQUID 5L



LIQUID 20L



SALT 1KG



SALT 5KG



SALT 20KG



Natural Product

is an Essential Oil supplement obtained from mixture of three plant oil extracts: **Lavender, Pine and Eucalyptus oils.**

Innovative technique is applied in extraction and evaluation of each plant and its composition. Several carriers, like minerals in feed premix, dilution in water, in salt have been used.

No toxicity effect of NG on embryonated eggs.

NG gave better protection against H5N1 infection in vivo than in vitro.

NG reduces H5N1 virulence.

The growth performance of both Control group and Treatment group are almost the same except few parameters. All the crucial parameters of both control and treatment have been found better in performance than the set standard.

"РИЯ ФАРМАСЬЮТИКАЛ"
ОБЩЕСТВО С ОГРАНИЧЕННОЙ ОТВЕТСТВЕННОСТЬЮ

УТВЕРЖДАЮ

Директор

ООО "РИЯ ФАРМАСЬЮТИКАЛ"

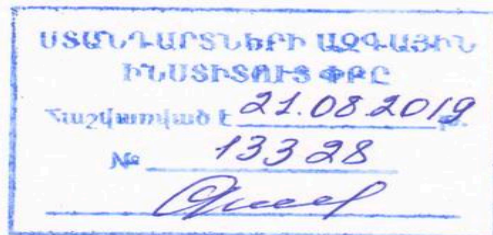


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APPROVED

By Y. A. Melkonyan,

Director

RHEA PHARMACEUTICAL LLC

/signature/

Seal

August 8, 2019

'Natural Guard'
FEED ADDITIVE
Specifications
ТУ АМ 50106084.8409 -2019
Introduced for the first time

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RESEARCH ARTICLE
Open Access

Natural oil blend formulation as an anti-African swine fever virus agent in *in vitro* primary porcine alveolar macrophage culture

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Abstract

Background and Aim: African swine fever is one of the severe pathogens of swine. It has a significant impact on production and economics. So far, there are no known remedies, such as vaccines or drugs, reported working successfully. In the present study, the natural oil blend formulation's (NOBF) efficacy was evaluated against ASFV *in vitro* using porcine alveolar macrophages (PAMs) cells of swine.

Materials and Methods: The capacity of NOBF against the ASFV was tested *in vitro*. The NOBF combines *Eucalyptus globulus*, *Pinus sylvestris*, and *Lavandula latifolia*. We used a 2-fold serial dilution to test the NOBF formulation dose, that is, 10⁵ HAD50/mL, against purified lethal dose of African swine in primary PAMs cells of swine. The PAM cells survival, real-time polymerase chain reaction (PCR) test, and hemadsorption (HAD) observations were performed to check the NOBF efficacy against ASFV.

Results: The *in vitro* trial results demonstrated that NOBF up to dilution 13 or 0.000625 mL deactivates the lethal dose 10⁵ HAD50 of ASFV. There was no HAD (Rosetta formation) up to dilution 12 or 0.00125 mL of NOBF. The Ct value obtained by running real-time PCR of the NOBF group at 96 h post-infection was the same as the initial value or lower (25), whereas the Ct value of positive controls increased several folds (17.84).

Conclusion: The *in vitro* trial demonstrated that NOBF could deactivate the ASFV. The NOBF has the potential to act as anti-ASFV agent in the field. The next step is to conduct *in vivo* level trial to determine its efficacy.

Keywords: African swine fever virus, *in vitro* trials, natural oil blend formulation, primary porcine alveolar macrophages cells.

Introduction

African swine fever virus (ASFV) reported as deadly for pigs. It is listed as a “notifiable disease” by the OIE due to high illness rates and a high mortality rate, up to 100%, and substantial financial losses [1-3]. Further spread of ASF to China has had disastrous consequences, especially instead of the fact that China contains more than half of the world’s pig population [4]. To date, as far as Vietnam is concerned, ASF has appeared in all 63 provinces of Vietnam, has destroyed more than 5.6 million pigs (more than 20% of total pigs), has decreased pork production by 8.3%, and has affected mainly small-scale farms [5-8].

The typical signs and symptoms of ASF are high fever, decreased appetite and weakness, difficulty

standing, red or blue blotches on the skin (particularly around ears and snout), and, especially in sows, the symptoms of miscarriage, stillbirths, and weak litters can occur [9,10]. Like, diarrhea, vomiting, and difficulty breathing or coughing, the symptoms can also occur with the disease [9]. ASFV is a large, enveloped and structurally complex DNA virus with the *Asfarviridae* family’s icosahedral morphology. The virus can persist for a long time in the environment, carcasses, and various swine products. The vectors and carriers of the ASF virus are warthogs (*Phacochoerus africanus*), bush pigs (*Potamochoerus porcus* and *Potamochoerus larvatus*), and soft ticks (*Ornithodoros moubata*) [4] in which the virus is transmitted trans-staidly and through transovarial routes [9].

The role of natural oils as antiviral components is well known. As a standardized compound, natural products are significant components with antiviral properties [11]. A formulation was developed by blending three natural oils, *Eucalyptus globulus*, *Pinus sylvestris*, and *Lavandula latifolia*, with antiviral properties. Cineole, the significant component of eucalyptus oil, has potent anti-inflammatory and

THU Y

Dịch tả heo châu Phi (ASF) và giải pháp ngăn chặn ở Việt Nam

ASF BÀ LÂY LAN NHANH VÀ XÂY RA Ở MỖI LOẠI HEO, MỌI LÚA TUỔI; VỚI TỶ LỆ CHẾT CÓ THỂ LÊN ĐẾN 100%, GÂY THIẾT HẠI KINH TẾ LỚN CHO NGƯỜI CHĂN NUÔI.

Nguyễn nhân

Bệnh do virus African Swine Fever gây ra. Khi vào cơ thể heo, virus di chuyển, tồn tại và phát triển ở mọi khi quan và mô bào của cơ thể. Chúng tồn tại và giữ nguyên độc lực tới 6 năm trong điều kiện lạnh, 4 - 5 tuần ở nhiệt độ phòng. Virus này có sức đề kháng rất mạnh với điều kiện tự nhiên. Bên cạnh đó, đường truyền lây ASF cũng rất đa dạng.

Triệu chứng

ASF biểu hiện ở 4 thể: thể cấp tính, thể cấp tính, thể mãn tính và thể bệnh ẩn. Thời gian ủ bệnh của thể cấp tính từ 5 - 7 ngày, heo sốt cao 41 - 42°C, da đỏ hoặc tím, xuất hiện dịch rỉ mắt và chảy dịch mũi. Vật nuôi kém ăn hoặc bỏ ăn, đi lã hoặc khàn, khớp không yếu ớt. Heo khó thở, chết đột ngột. Virus xâm nhập vào đường tiêu và vật gây nên những vết xuất huyết ở ngoài da, mắt. Nếu giải phẫu, phát hiện được ruột bị xuất huyết; phổi, lách, thận bị sưng.

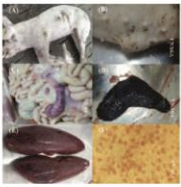
Nếu giải phẫu, phát hiện các hạch lympho sưng, xuất huyết; lách sưng, xuất huyết và xuất huyết; phổi sưng huyết, xuất huyết điểm, phù nề phổi và phế nang; gan và túi mật sưng, xuất huyết; thận xuất huyết điểm và thận và xuất huyết ở nhu mô thận.

Giải pháp từ sản phẩm NATURAL GUARD

Khoa Thú y, Học viện Nông nghiệp Việt Nam kết hợp nhóm chuyên gia nghiên cứu của PT. Adecplus Pharmaceutical Sciences Indonesia đang tiến hành nghiên cứu, thí nghiệm trên quy mô phòng thí nghiệm cho sản phẩm NaturalGuard (NG) với đặc tính kháng virus gây bệnh ASF. Sản phẩm này ra trước vòng lã cho người chăn nuôi.

Phương pháp thí nghiệm

Các thí nghiệm *in vitro* và *in vivo* sử dụng sản phẩm NG đã được tiến hành để chứng minh khả năng kháng virus ASF. Các thí



Một số hình ảnh trên chúng tôi cũng có thể thấy các heo bị nhiễm ASF đang nằm và heo PAMs đã chết đang được ăn vờng gây nhiễm và virus dịch tả heo châu Phi (ASFV). Ở heo có triệu chứng heo nóng, có giải trước khi chết. (B) hoạt huyết điểm mắt da heo sau heo nóng, sau ngày 01 heo nóng heo rút sưng, xuất huyết. (C) Lách sưng, nhũ huyết. (F) Virus ASF nhũ heo trên tế bào PAMs và các rosettes lách đang ăn vờng



NATURAL GUARD: Improve Poultry, Swine and Cattle Health

Kết quả nghiên cứu cho thấy sản phẩm không độc cho heo và virus gây bệnh bị tiêu diệt hoàn toàn trong cơ thể, heo nóng khỏe mạnh và không phát hiện virus ASF trong ruột 2 tháng sau khi gây nhiễm.

nghiệm *in vitro* đã được thực hiện trên tế bào đại thực bào phổi heo (PAMs), và được coi là nhũ huyết; phổi sưng huyết, xuất huyết điểm, phù nề phổi và phế nang; gan và túi mật sưng, xuất huyết; thận xuất huyết điểm và thận và xuất huyết ở nhu mô thận.

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Research Article

EFFICACY OF NATURAL HERBAL FORMULATION "NATURALGUARD" AS AGP REPLACEMENT ON SWINE PRODUCTIVITY IN INDONESIA

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INTRODUCTION

Excessive utilization of Antibiotic and Growth Promoter (AGP) in animal farming stimulates the bacterial resistance. The AGP residue in animal products (meat, milk, and egg) is harmful to human health. The European Union has banned the utilization of AGP in animal farming since 2006. The government of Indonesia has banned the uses of AGP in livestock feed since 2018. The natural oils are one of the potential agents to replace the uses of antibiotics in the feed.

It is a need of an hour to work on the development of a substitute for AGP in order to increase the productivity and to prevent various types of diseases. Essential oils are generally recognized as safe and recognized by the Food and Drug Administration (FDA). Essential oils can inhibit the growth of pathogen microbes in intestines and improve nutrient digestibility.

Natural guard (reg no Ty AM 50106084.8409-2019 in Armenia) is a feed additive containing three essential oils consisting of Pine oil, *Eucalyptus globulus* oil, Lavender oil. The Natural guard acts as an immunomodulator that not only improves productivity but also expected to improve the quality of the pork. The present study was conducted to evaluate the efficacy of Natural guard as a potential agent to replace the AGP in swine feed.

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MATERIALS AND METHODS

Experimental design and animals

A 90-day growth assay was performed to evaluate the efficacy of Natural guard on the productivity and blood parameters of the piglet. A total of 80 crossbred piglets (Yorkshire) i.e. 40 piglets of similar age, weight, and sex in each group, were assigned to two dietary treatments, control with Antibiotics and Growth promoter and without Natural guard supplement and treatment with Natural guard supplement (2 kg/ton feed) and without Antibiotics and Growth promoter. The experiment consisted of a randomized complete block design with two replicates of the dietary treatments.

The piglets were fed twice daily. Throughout the experiment, all piglets had *ad libitum* access to drinking water. Pigs were weighed on monthly (day 0, day 30, day 60 and day 90) basis and feed consumption was determined on a weekly basis. At the end of the experiment, blood samples were collected with a monoxet standard hypodermic needle. Blood samples were collected in 3 ml tubes containing ethylenediaminetetraacetic acid (EDTA). Blood samples were used to study the blood biochemical and hematological parameters of experimental swine.

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Research Article

Effect of Natural Oil Blend Formulation (NOBF) on Milk Production and Productivity in Dairy Cattle

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Abstract

Background and Objective: The effect of a blend of three essential oils, eucalyptus oil, pine oil and lavender oil, was investigated on dairy cows performance. The trial's main objective was to evaluate the performance of Natural Oil Blend Formulation as antibiotics and growth promoter replacement by maintaining the milking quality and quantity. **Materials and Methods:** Thirty Holstein-Friesian crossbred lactating cows, averaging 514 kg bwt, were assigned into two groups, 15 cows in each group. The experiments lasted for ten weeks, with the first two weeks as the adjustment period, followed by eight measurement weeks. All cows were weighed at the start and end of the experiment. Blood samples were collected from the caudal vein at the end of the trial. The parameters studied were lactation dairy cattle on dry matter intake, body weight and milk production. **Results:** The trial outcome stated that the addition of Natural Oil Blend Formulation (NOBF) has no adverse effect on Dry Matter Intake (DMI), Average Daily Gain (ADG) and Fat Corrected Milk (FCM) 4%. However, cattle in the treatment group tend to be higher in DMI (0.51 kg, p = 0.12) and higher in ADG (0.19 kg, p = 0.11). The blood biochemical were unaffected by treatment. However, calcium tends to be higher (p<0.1) compared to control. **Conclusion:** In conclusion, NOBF supplementation may improve milking cows' feed utilization and performance, hence, the underlying mechanisms leading to this improvement merit further investigation.

Key words: Natural oil blend formulation (NOBF), milking cows, productivity, feed utilization, ruminant livestock, milking machines, dry matter intake

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.



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MEEGID XV 15th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Disease – MEEGID XV (Online and On-Demand) Submission ID 29 Title Natural oil blend formulation (NOBF) protects Penaeus vannamei Boone, 1931 from white spot syndrome virus (WSSV) and enhances the productivity in the culture ponds Abstract White spot syndrome virus (WSSV) is lethal in penaeid shrimp. Successful efforts were made to develop a natural oil blend formulation (NOBF) with anti-WSSV properties using Eucalyptus globulus, Pinus sylvestris, and Lavandula latifolia in an equal proportion of water at a ratio of 1:1. A bioassay challenge trial was conducted using 1 g of 144 specific pathogen-free Penaeus vannamei Boone, 1931 samples in 4 aquarium replicates for each group. A NOBF dose of 0.2 ppm was applied throughout the trial period by mixing in aquarium water daily, starting seven days before challenge. The efficacy of NOBF against WSSV was measured using a modified per os method of challenge demonstrated in the current work. The cumulative mortality in the positive control group reached 89.6 % ten days post challenge. NOBF was also applied in six commercial shrimp ponds in a WSSV-prone area in East Java, Indonesia. Each pond was of equal size, 1000 m² and 1.2 m depth. The pathogenic Vibrio count of pond water was acceptable (<1000 cfu.mL⁻¹). The NOBF-applied ponds had better productivity (control 14.239 ton.ha⁻¹ and NOBF ponds 15.421 ton.ha⁻¹). The trial outcomes show that NOBF is safe and user-friendly, with properties that reduce WSSV load.

MEEGID XV 15th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Disease – MEEGID XV (Online and On-Demand) Submission ID 28 Title Identification of African Swine Fever Virus DNA polymerase X potential inhibitors in Natural oil blend formulation by Structure-Based Virtual Screening Approach. Abstract In this study, virtual screening approach was used to investigate Natural oil blend formulation (NOBF) individual compounds against African Swine Fever Virus (ASFV) DNA polymerase X (DNApolX). Using a combination of bioinformatics and computational tools, we predicted the interaction of dGTP binding pocket in the active site of ASFV DNApolX with 9 known constituted small molecules of blended essential oil formulation as well as natural ligand dGTP and known ASFV replication inhibitors rosetta and rosetta-2. The reference compounds, we found that all studied small molecules interacted with common amino acid residues in the dGTP-binding pocket of ASFV DNApolX with high docking score and HF score value. hotspot residues of the enzyme (Fig. 4). Fig. Molecular interactions of reference molecules dGTP, oxyclohexanol and rosetratol with ASFV DNApolX hotspot residues. Superimpositions of hotspot amino acid residues on the binding site structure-based model of the ASFV DNApolX and surface exposed amino acid residues are shown. Fig. 2D Interactions of dGTP with ASFV DNApolX. dGTP interacts with the hotspot residues of the PolX and forming van der Waals bonds with Val37 and Phe116 surface exposed hotspot amino acid residues. Unfavorable donor-donor and alkyl interactions formed with Ser99 and Val120.

MEEGID XV 15th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Disease – MEEGID XV (Online and On-Demand) Submission ID 30 Title Development of essential oil blend formulation as a disinfectant against African Swine fever virus (ASFV) agent in PAM cells of Swine Abstract African swine fever virus has a significant impact on swine production and the economics of the swine-producing countries. The role of strict biosecurity measures becomes critical when there are no known remedies yet. Disinfection is considered a significant part of biosecurity measures and plays a vital role in reducing the risk of contaminating the environment. Successful efforts have been made to optimize a formulation Essential Oil Blend (EOB) product to determine its efficacy against the African swine fever virus (ASFV) in vitro conditions. The Essential Oil Blend (EOB) comprises a blend of, i.e., Eucalyptus oil, Gardenia oil, and Jasmine oil. The in vitro trial results demonstrated that EOB up to dilution ten could degenerate the lethal dose log 5 of ASFV. The work was shown by observing hemadsorption (Rosetta formation) and conducting a real-time PCR test. There was no Rosetta formation up to dilution 11 of EOB. The Ct value of the EOB group at 96 hours post-infection (hpi) was the same as the initial value or lower (25) than it, whereas the Ct value of positive control increased several folds (17.84). It is a potential water supplement to work against ASFV and enhance pig immunity to fight against common pathogens.

INTRODUCTION: AFRICAN SWINE FEVER (ASF) IS CAUSED BY AFRICAN SWINE FEVER VIRUS (ASFV) THE VIRAL GENOME... RESULTS & DISCUSSION: Molecular docking... CONCLUSIONS: The present study... METHODS: The present study... [P.02] Identification of African Swine Fever Virus DNA Polymerase X Potential Inhibitors in Natural Oil Blend Formulation (NOBF) by Structure-Based Virtual Screening Approach. Presenter: Rajeev Kumar Jha

06. Virus Evolution 14:30 - 15:30. Chair: Martine Peeters. Speakers: Rajeev Kumar Jha, Takuya Kawahata, Mariana Kikuti, Maylis Layan, Marie Claire Morley, Alessandra Mozzi.

Posters. [P.03] Development of essential oil blend formulation as a disinfectant against African Swine fever virus (ASFV) agent in PAM cells of Swine. Presenter: Rajeev Kumar Jha.

[P.03] Development of Essential Oil Blend Formulation (EOBF) as a Disinfectant against African Swine Fever Virus (ASFV) agent in PAM cells of Swine. Presenter: Rajeev Kumar Jha. RESULTS AND DISCUSSION: The African Swine Fever Virus (ASFV) is considered one of the most... CONCLUSION: The obtained results demonstrated that EOB up to dilution ten could degenerate the lethal dose log 5 of ASFV.





**Naturalguard as essential oil blend supplement to
reduce the disease risk and increase the
productivity of Swine**

Rhea Natural Sciences

NATURALGUARD INTRODUCTION



- *Product Introduction and formulation*
- *Mode of Action*
- *Characteristics*
- *Application method and dose*

FIELD OBSERVATION



- *Trials conducted in Contract farm, CPI, Bali*
- *Trials conducted in a farmer's farm, Solo, Indonesia*
- *Trials conducted in a farm, Vietnam*

LABORATORY REPORT



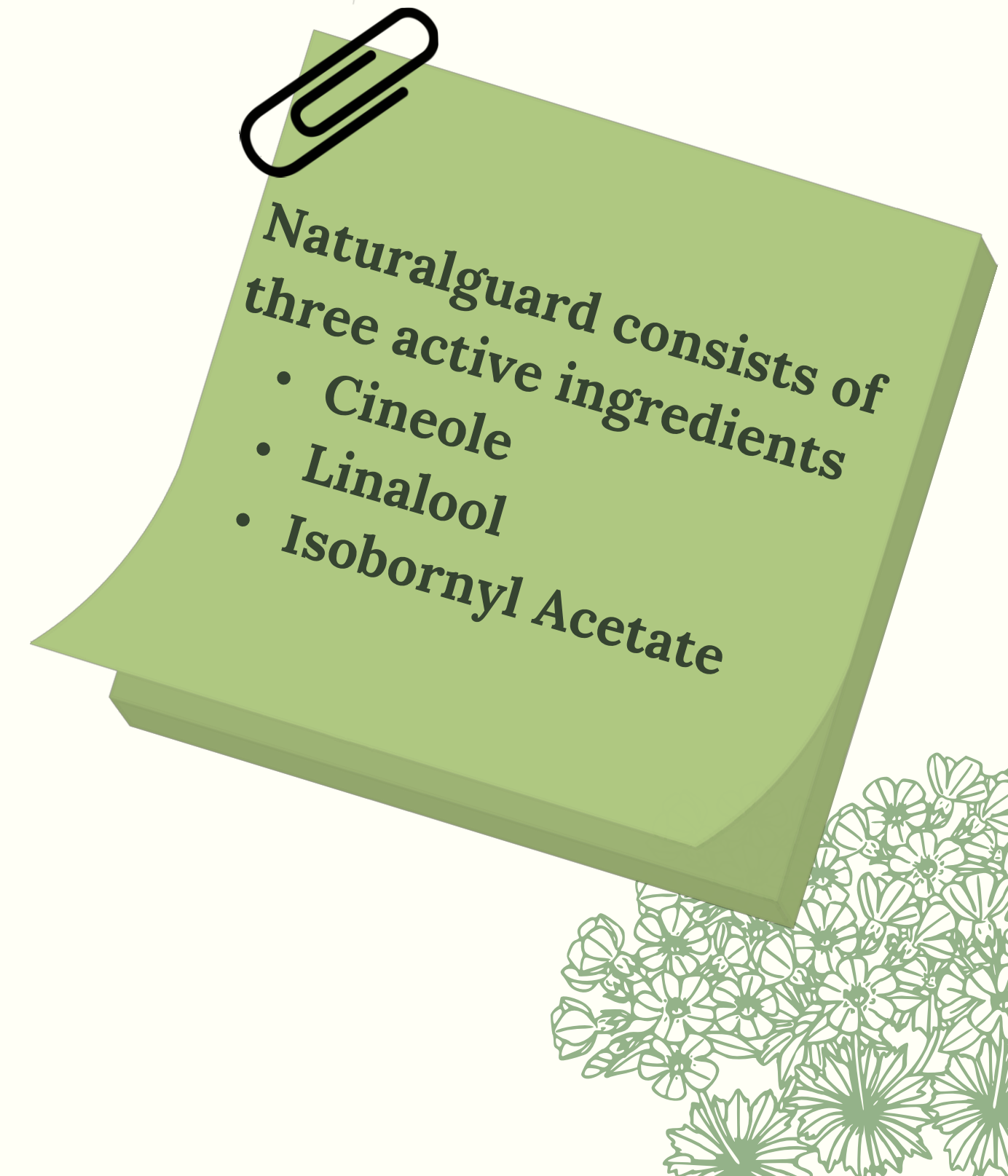
- *In vitro trials of Naturalguard antiviral activity*

CONCLUSION



- *Efficacy of Naturalguard*

- Naturalguard, containing Essential oils, possesses pronounced capability to stimulate the induction of humoral and cell-mediated immune response of lymphocytes to the antigens of pathogenic bacteria and alloantigens.
- It maintains the immunity level by keeping IgG levels high, which helps to protect the animal from infectious diseases.
- It reduces the mucosal immune response, which can be seen by low IgM levels in the NG treated group.
- It maintains the essential metabolic functions of an animal's body and reduces the stress level.
- It acts directly against specific pathogens like African Swine Fever and pathogenic bacteria.



NATURALGUARD FORMULATION

The selection of blend oils was carried out based on anti-viral -and immunomodulating properties, as well as their compatibilities. The targeted active ingredients are as follows:

- Cineole extracted from *Eucalyptus globulus*
- Isobornyl acetate extracted from *Pinus sylvestris*, and
- Linalool extracted from *Lavandula latifolia*

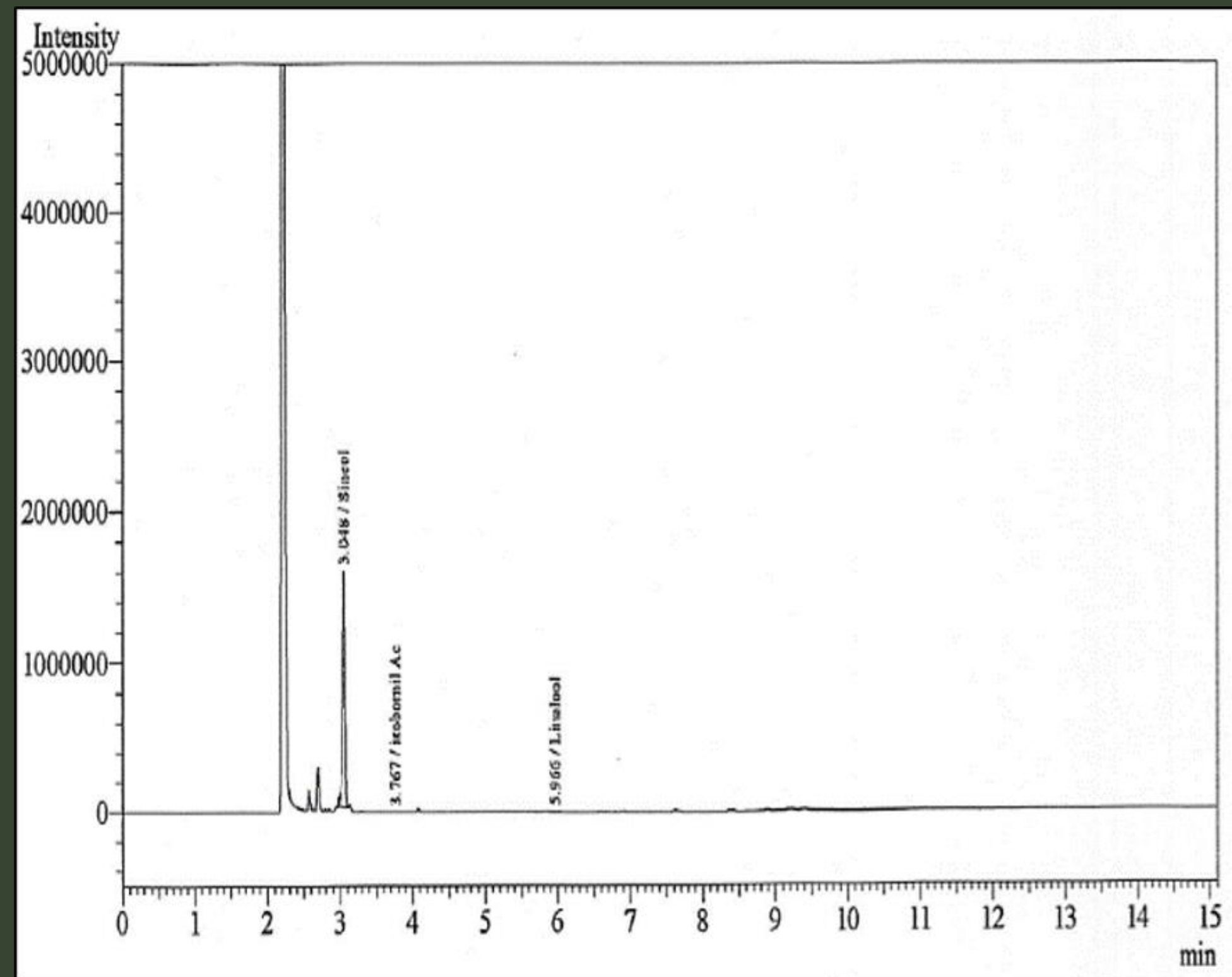


Figure: Chromatogram of complete NOBF compound's peak i.e. Cineole, Linalool and Isobornyl acetate

CHARACTERISTICS OF NATURALGUARD



Mix recommended
dose of
Naturalguard in 10
liters of clean water.

Mix well
for 2 to 3
minutes.

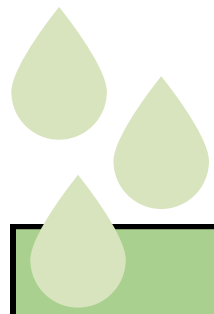
Mix into the drinking
water tank



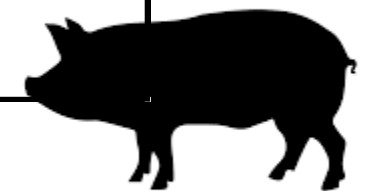
NATURALGUARD APPLICATION in PIG FARM



RECOMMENDED DOSE



Application Method		Dose	Frequency
Mix in the feed	Preventive	2 kg per ton feed	Daily
	Treatment	4 kg per ton feed	Daily
Mix in the drinking water	Preventive	80 ppm	Daily
	Treatment	160-240 ppm	For 7-10 consecutive days



Summary of Laboratory level trials and field observation

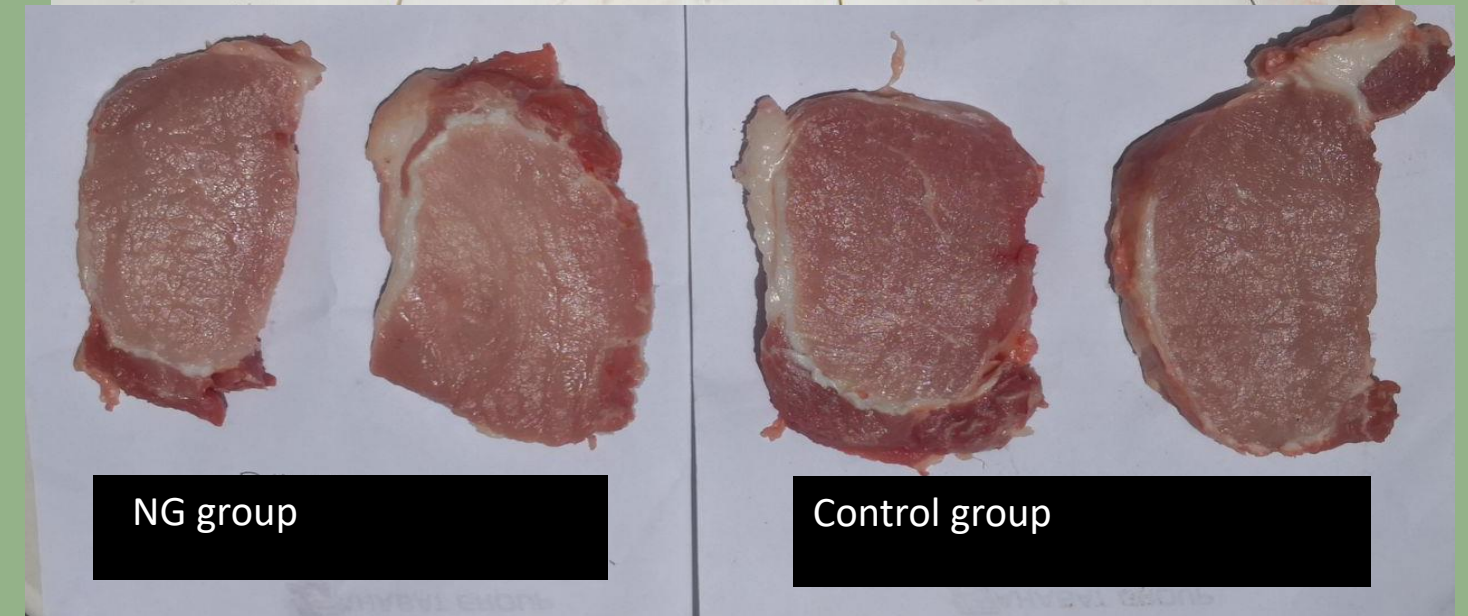
Beneficial Properties	Test Methodology	Results
Naturalguard as Antibiotics and Growth Promoter replacement in pig feed	Commercial farms, CPI, Indonesia	Growth performance and survival rate (up to 5 %) increased in Pigs. Higher protein content and lower cholesterol content in NG fed meat of Pigs.
Antibiotics and Growth Promoter replacement	Commercial farms, UGM, Indonesia	Growth performance and survival rate increased
Performance and blood quality improvement	Commercial farms, UGM, Indonesia	Higher weight gain, lower FCR. Higher protein content, Lower Uric acid and triglycerides and lower cholesterol content in NG fed meat of Pigs.
Anti-ASFV	In Vitro , VNUA, Vietnam	Effective, 100 % deactivation of log 5 ASFV in PAM cells
Anti-ASFV	In Vivo, VNUA, Vietnam	Effective, 100 % effective in preventing horizontal transmission
Anti-ASFV in commercial Sow farms, Indonesia	Commercial farms, CPI, Indonesia	Effective, 80 % effective in preventing horizontal transmission

Serial No.	Performance Parameters	Naturalguard	Control
1.	No. of pigs	168	168
2.	Av. Initial Body weight (kg)	10.57 kg	10.50 kg
3.	Average rearing period	144 days	144 days
4.	Percentage mortality	3.6 %	6.1 %
5.	Average Body weight at harvest (kg)	112.3 kg	112.71 kg
6.	Average Daily weight gain (ADG) (Kg/day)	0.70	0.70
7.	Actual FCR	3.029	3.035

- The *in vivo* level trial was established to determine the role of Naturalguard as antibiotics and growth promoter replacement in the pig feed.
- The results showed that pigs on Naturalguard performed better in terms of the same ADG, lower FCR, and better survival rate.

Meat sample analysis (Loin portion)

Meat analysis	NG	Control	P value
Protein (%)	22.97	21.62	0.098
Ash (%)	1.41	1.13	0.335
Cholesterol (mg/100 g)	54.78	56.59	0.726
Fat (%)	5.73	6.45	0.466
Energy from Fat (Kcal/100 g)	51.57	58.01	0.466
Total Energy (Kcal/100 g)	156.66	172.51	0.287
Fiber (%)	0.62	0.61	0.851
Carbohydrate (%)	3.30	2.54	0.465
Moisture (%)	66.52	66.28	0.746



- The cholesterol and triglyceride levels of the Naturalguard group (161.81 mg/dL) were lower than control (175.02 mg/dL).
- The Uric acid level of Naturalguard fed pigs (1.78 mg/dL) were lower than control(2.92 mg/dL).
- The obtained results showed that Naturalguard is a potential candidate to replace AGP in pig feed.
- NG pork had higher tender percentage (64.4%) than control.
- NG pork was more delicious (57.7%) than control.

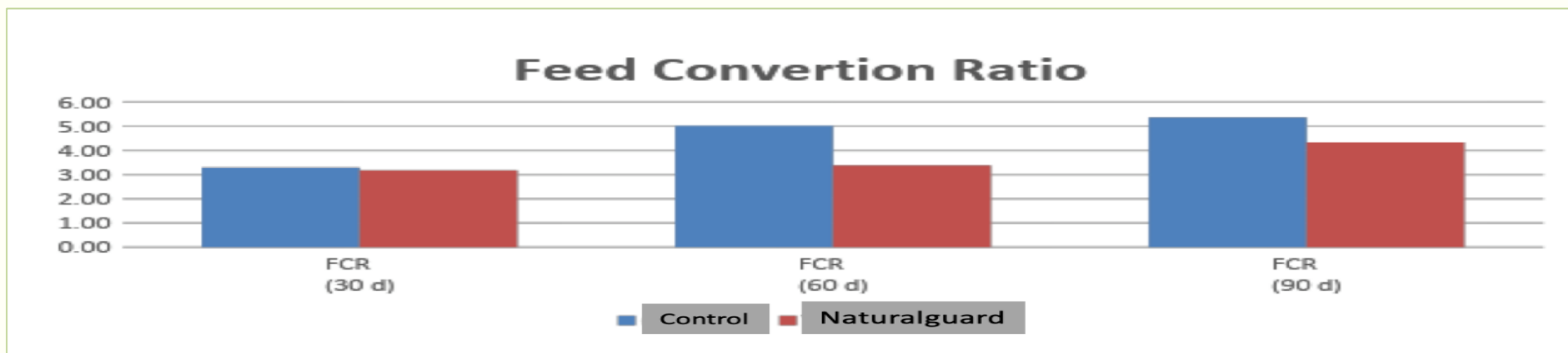
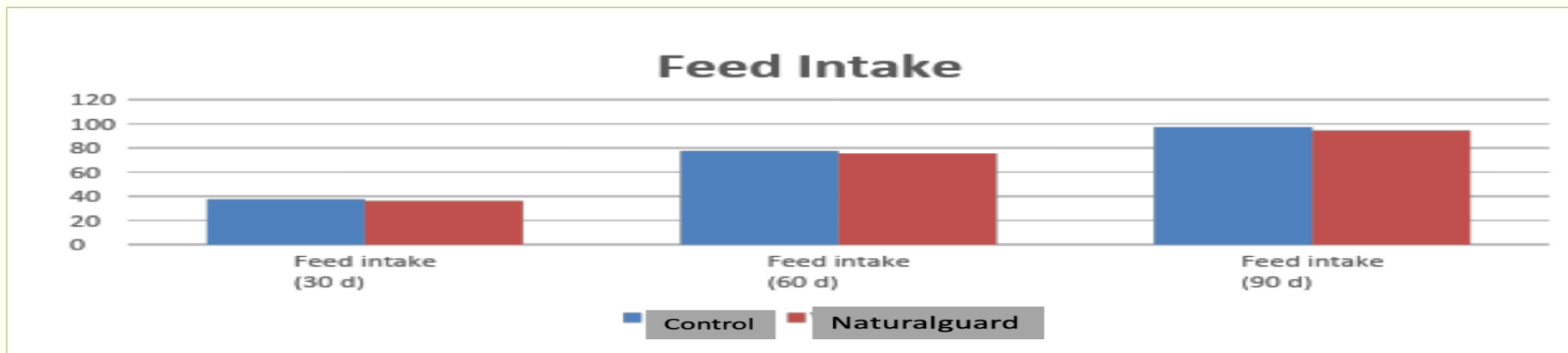
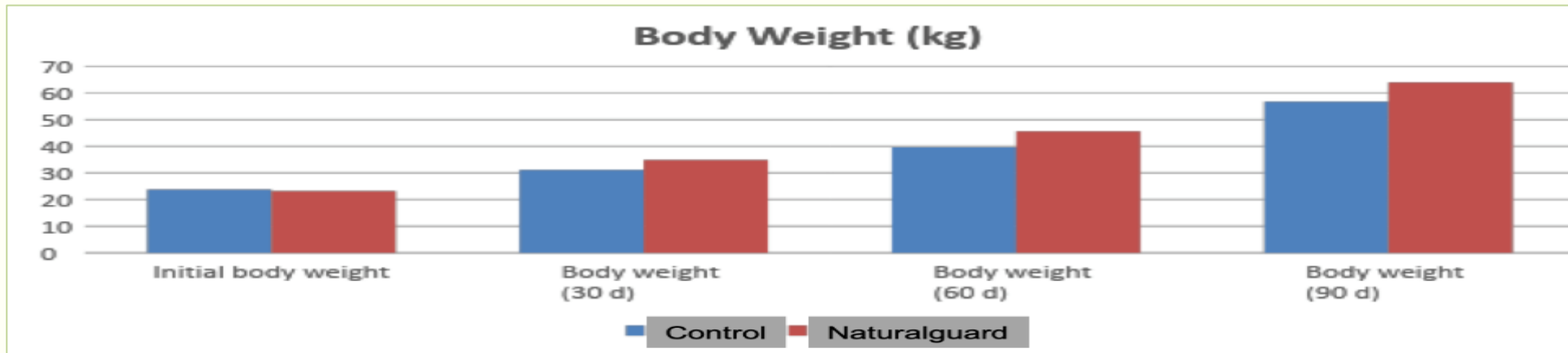
Surveillance of mortality and disease incidence in Naturalguard applied Contract farm, CPI, Bali

Summary Trial NG and EG														Year 2020	
No.	Type	Red Zone Date (Red/Green) (From News)	Pig Stock (heads)	NG&EC	Start Date NG&EG	ASF	Start Clinical Sign Date	Mortality		End Stock	% Mortality		Remark		
								Dead	Cull		Dead	Cull			
1	MW	Apuh	Jun-20	106	YES	10-Mar-20	V			106	0%	0%			
2	MW	Apuh	Jun-20	105	YES	10-Mar-20	V			105	0%	0%			
4	MW	Cau Tua	Feb-20	66	YES	10-Mar-20	V			66	0%	0%			
5	MW	Cau Tua	Mar-20	56	YES	10-Mar-20	X	18-Mar-20	38	18	0	68%	32%	5th (The same Area with Dio)	
6	MW	Cau Tua	Mar-20	74	NO=>YES	10-Mar-20	X	01-Mar-20	53	21	0	72%	28%	4th Out Broken (Too late to treat by NG)	
9	MW	Pengotan	Feb-20	69	YES	10-Mar-20	V			69	0%	0%			
10	MW	Pengotan	Feb-20	81	YES	10-Mar-20	V			81	0%	0%			
11	MW	Pengotan	Feb-20	77	YES	10-Mar-20	V			77	0%	0%			
12	MW	Pengotan	Feb-20	66	YES	10-Mar-20	V			66	0%	0%			
13	MW	Pengotan	Jun-20	62	YES	10-Mar-20	V			62	0%	0%			
15	MW	Pujung	Mar-20	99	YES	10-Mar-20	X	May-20	18	81	0	18%	82%	6th	
16	MW	Sandan		87	YES	10-Mar-20	V			87	0%	0%			
17	MW	Taro	Apr-20	80	YES	10-Mar-20	V			80	0%	0%			
18	MW	Taro	Apr-20	69	YES	10-Mar-20	V			69	0%	0%			
19	MW	Taro	Apr-20	94	YES	10-Mar-20	V			94	0%	0%			
20	MW	Temasi	Mar-20	87	YES	10-Mar-20	V			87	0%	0%			
MW		16		1,278					109	120		18%			
		3													
		19%													

V	ZONE Free
X	ZONE Out break

- Naturalguard at the dose of 240 ppm in drinking water daily was applied in Twenty saw farms in ASF red zone area.
- 18 farms had a usual crop.
- Two farms detected mortality and infection, which later found that infection occurred within two weeks of NG application.
- The obtained field data showed that NG is effective against unknown pathogens and also helpful in preventing horizontal transmission so controls the spread of infection.

Naturalguard-powder on the Productivity and Blood Parameters of Swine by UGM, Indonesia



Feeding trial for piglet start from 1 month age :

- Naturalguard feed (basal diet + NG Salt 2 kg/ton feed), n= 40 piglet
- Control (basal diet), n= 40 piglet

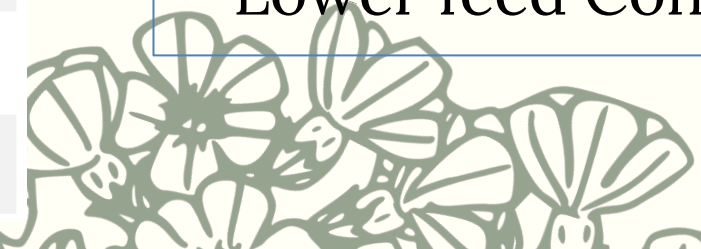
Variable measured

- Piglet growth performances (birth weight, weaning weight, body measurement, mortality, morbidity)
- Serum parameters
- Blood content : red blood cell, white blood cell, Packet cell volume, haemoglobin,
- Mineral (Ca, P, Mg), cholesterol, glucose, triglyceride, total protein, albumin, urea, creatinine.

Observations

- Improved body weight gain
- Decreased total feed intake
- Lower feed Conversion Ratio (FCR)

Treatment	Protein (g/dL)	Creatinine (mg/dL)	Uric Acid (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)
Control	6.79	1.43	2.92	175.02	36.19
Naturalguard	6.63	1.37	1.78	161.81	35.43
P Value	0.553	0.764	0.470	0.260	0.916



In vitro trials of Naturalguard antiviral activity against African swine fever virus strain, Vietnam

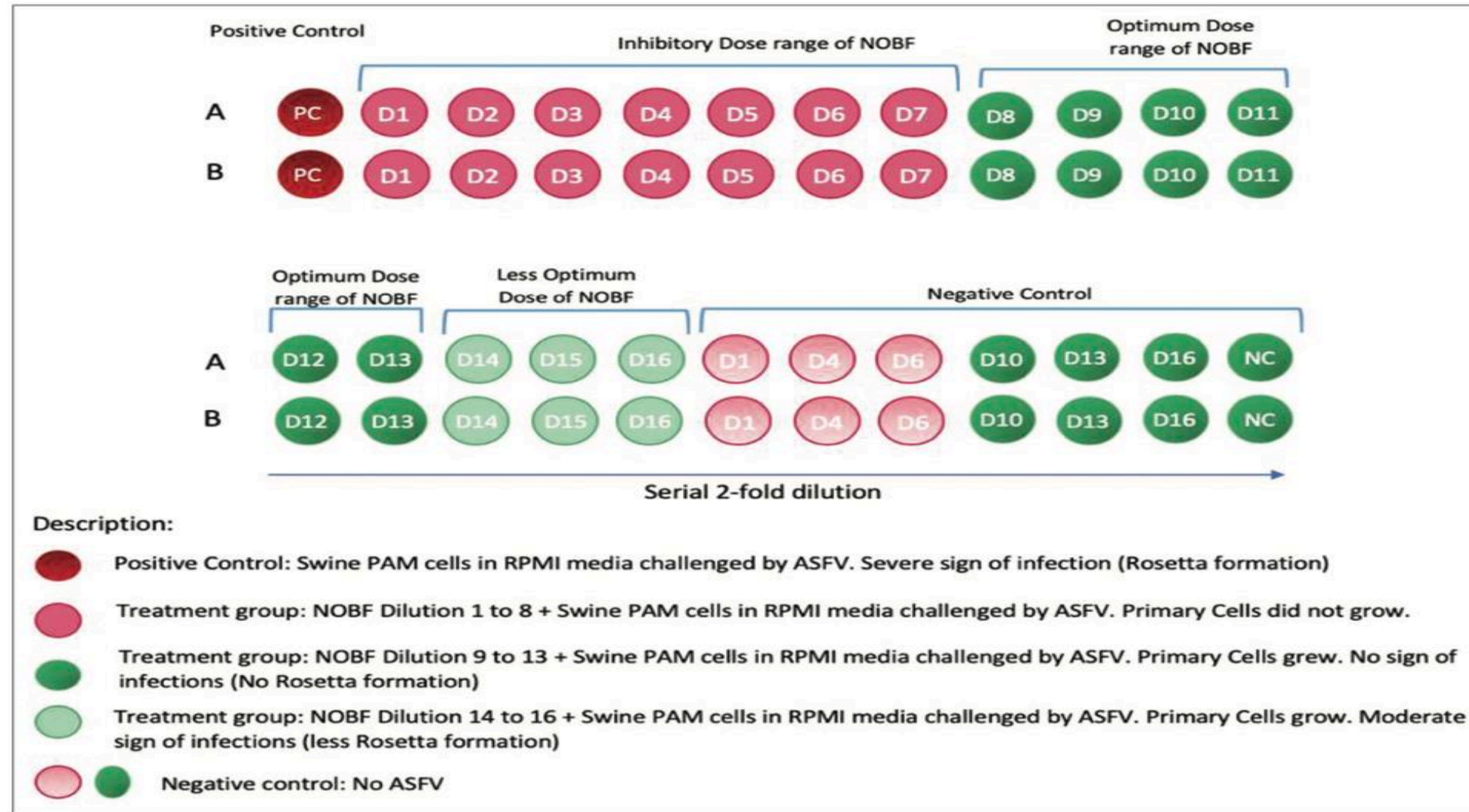
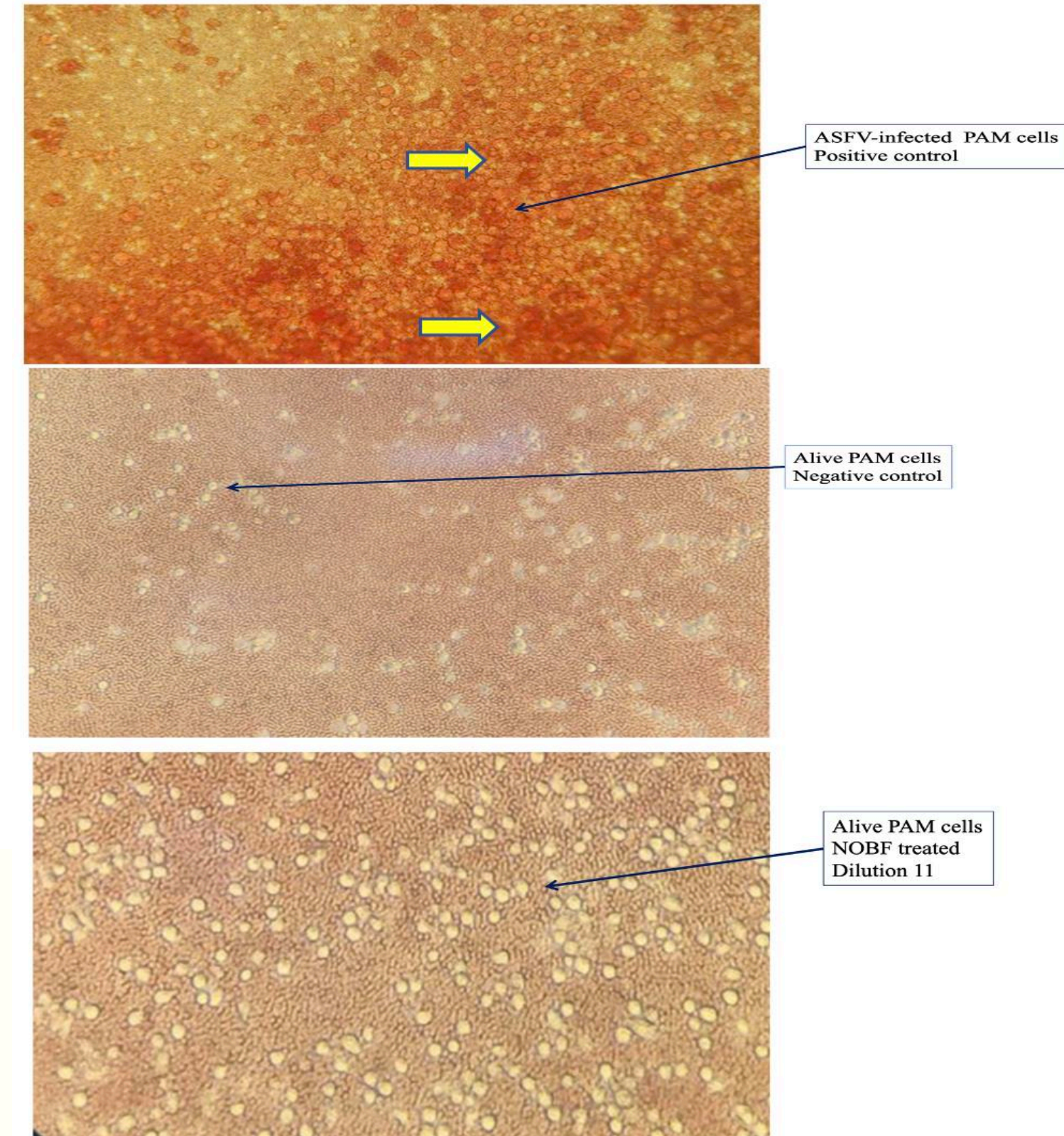


Figure-3: The illustration shows the result outcome of *in vitro* test. The inhibitory dose of the natural oil blend formulation (NOBF) was observed from dilution 1-7. The optimum and effective dose of NOBF against African swine fever virus (ASFV) was observed from dilutions 8-13. The less effective dose started from dilutions 14. The different dilutions of negative control (without ASFV) indicated the inhibitory and not-inhibitory doses of NOBF on porcine alveolar macrophages cells.

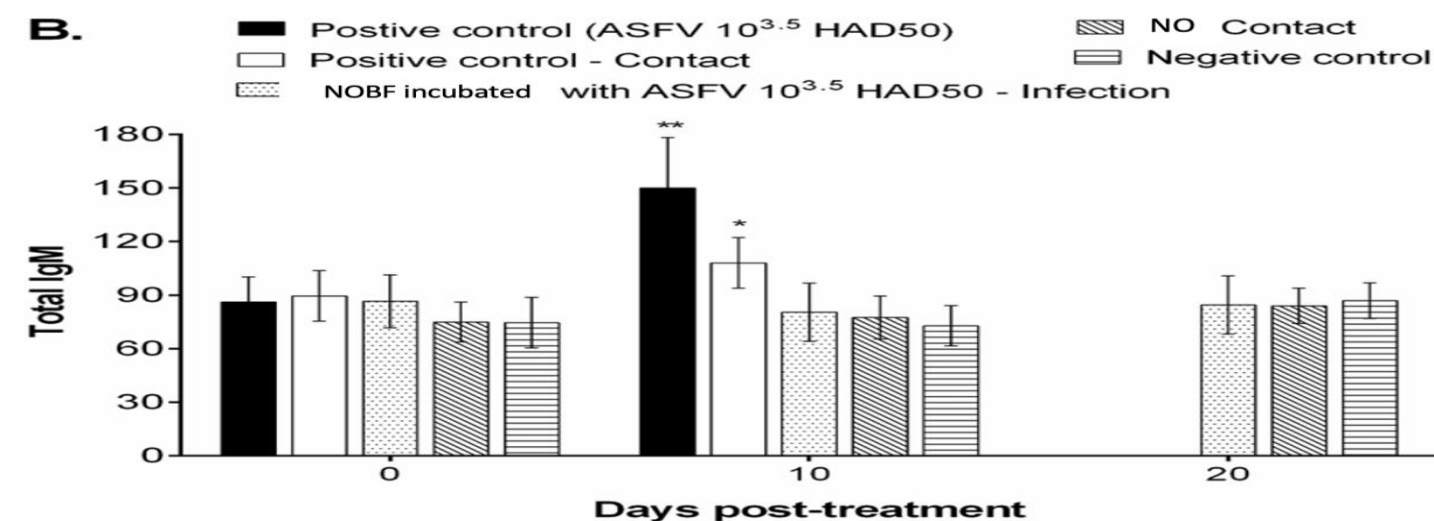
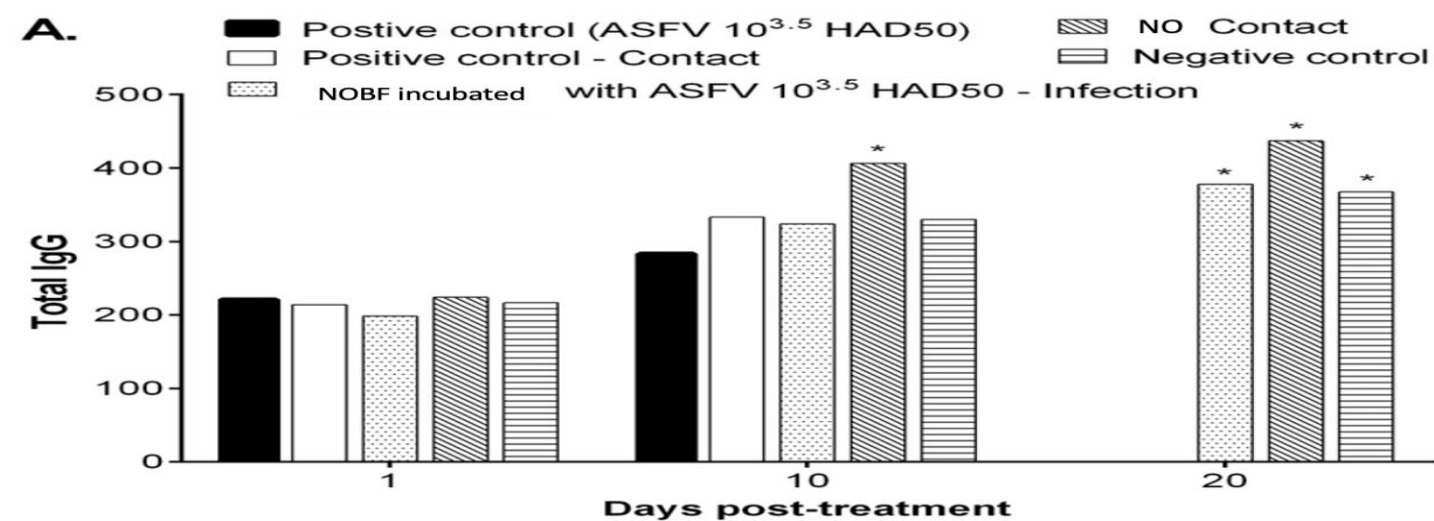


- The in vitro level trial was established to determine the efficacy of Naturalguard against lethal (log 5) and sub-lethal (log 4) doses of ASFV in porcine alveolar macrophages (PAM) cells.
- The obtained results from RT-PCR and HAD or Rosetta formation showed that Naturalguard was effective up to dilution 13 or 62.5 ppm to inhibit ASFV growth.

In vivo trials of Naturalguard antiviral activity against African Swine Fever Virus strain, Vietnam

Day of challenge/ Tag no	Negative Control			NOBF (Direct challenge)						NOBF (Incubated challenge)						Positive control					
	No challenge			Challenge			Cohoused			Challenge			Cohoused			Challenge			Cohoused		
	45	46	47	100	73	81	76	77	79	50	51	52	53	54	55	67	89	96	72	75	97
D-0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
D-10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
D-12	ND	ND	ND	34.62	32.15	34.76	ND	ND	ND	ND	ND	ND	ND	ND	ND	27.56	29.67	32.06	ND	ND	ND
D-14	ND	ND	ND	30.91	20.85	21.66	ND	ND	ND	ND	ND	ND	ND	ND	ND	16.51	17.21	23.38	ND	ND	ND
D-16	ND	ND	ND	19.25	15.98	17.91	ND	ND	ND	ND	ND	ND	ND	ND	ND	15.41	16.23	17.52	ND	35.95	ND
D-18	ND	ND	ND	24.17	-	16.75	ND	ND	ND	ND	ND	ND	ND	ND	ND	15.03	15.19	16.05	35.22	33.56	34,83
D-20	ND	ND	ND	22.44	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	-	-	15.03	24.52	23.19	27,83

Ct value ≥ 37 negative real-time PCR; ≤ 36 = Positive real-time PCR; ND (not detected) = Negative real-time PCR. ASFV = African swine fever virus, NOBF = Natural oil blend formulation



- The *in vivo* trial was established to determine the efficacy of Naturalguard against sub-lethal (log 4.5) doses of ASFV in pigs.
- The obtained results from RT-PCR and clinical symptoms, cumulative mortality, showed that Naturalguard effectively inhibited ASFV infection.
- The incubated challenged group showed that Naturalguard could deactivate the ASFV in direct contact.
- The RT-PCR data showed that Naturalguard prohibited the horizontal transmission of ASFV.
- The IgG levels in pigs which consumed Naturalguard were higher than in the control groups.
- The IgM levels in pigs which consumed Naturalguard were lower than in the control groups.

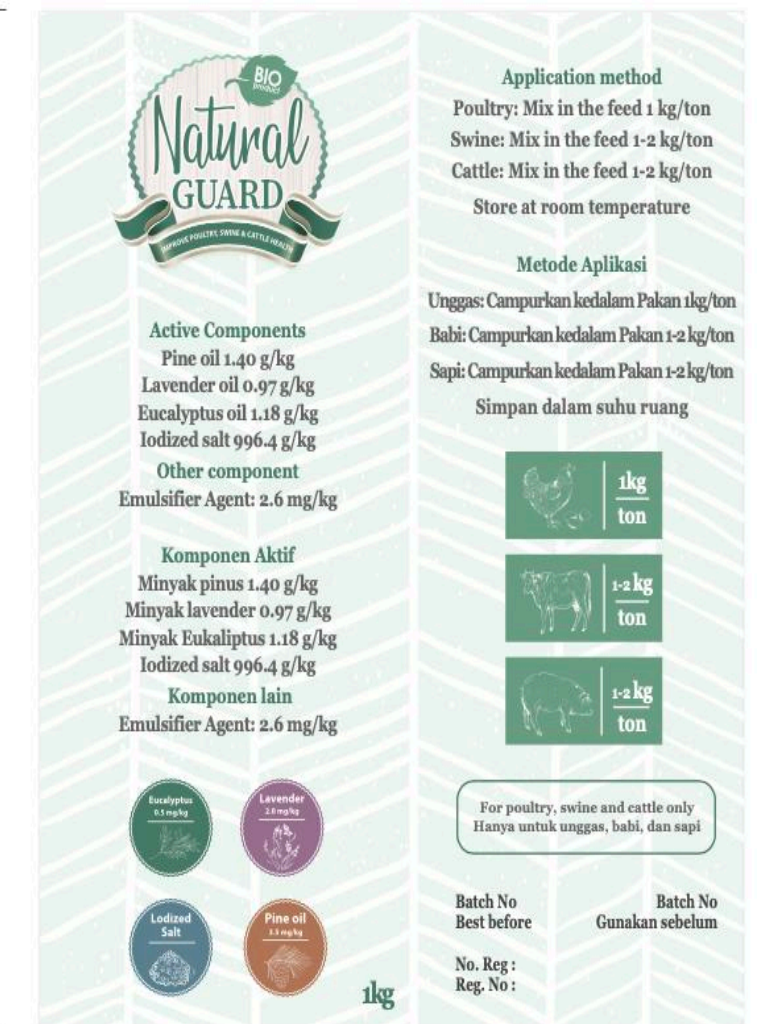
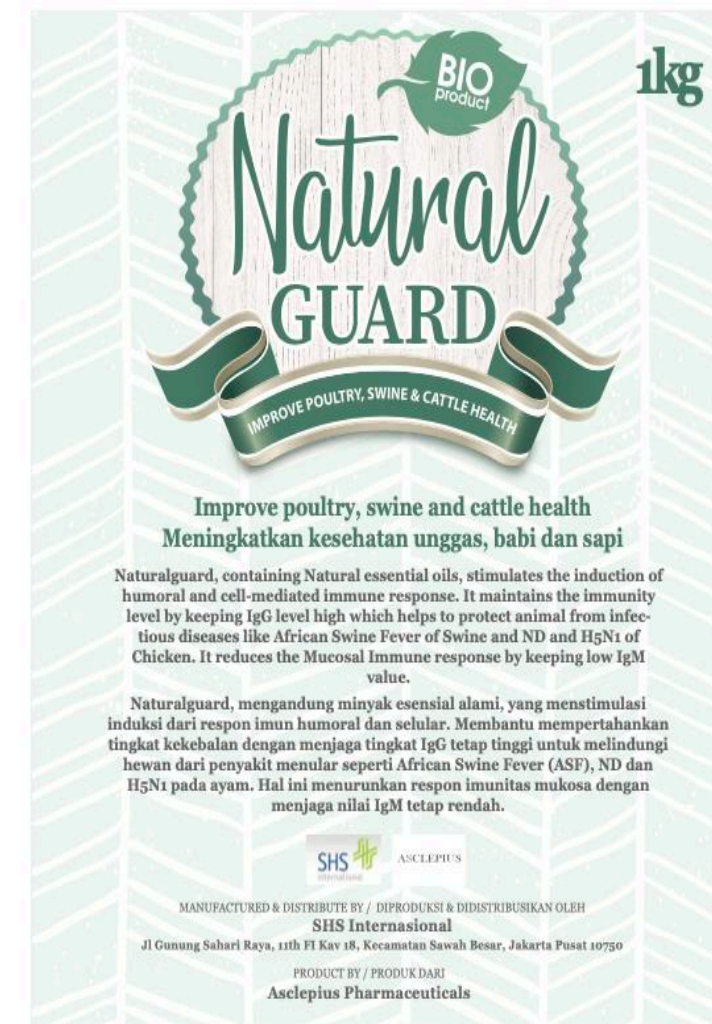
CONCLUSIONS

The developed blend formulation Naturalguard is an effective immunomodulator and anti-pathogenic agent which supports optimal pig growth resulting in better productivity.





Salt Pack 1 KG
CMYK 14cm X 17cm



NaturalGuard

We protect your Farm, Naturally



LIQUID 1L



LIQUID 5L



LIQUID 20L



SALT 1KG



SALT 5KG



SALT 20KG



Natural Product

is an Essential Oil supplement obtained from mixture of three plant oil extracts:
Lavender, Pine and Eucalyptus oils.

Naturalguard is blend of Lavender oil, Eucalyptus oil and Pinus oil.

It maintain the health of Pig, Poultry and large animals by keeping the immunity at optimum level.

It maintains the basic metabolic function.

It reduces the load of virus (ASF and H5N1 and) and other pathogens.

It possesses pronounced capability to stimulate the induction of humoral and cell-mediated immune response of cultured human lymphocytes to the antigens of pathogenic bacteria and alloantigen.

It increases the humoral immune response characterized by higher IgG level.

It reduces mucosal immune response characterized by lower IgM level.



"РИЯ ФАРМАСЬЮТИКАЛ"
ОБЩЕСТВО С ОГРАНИЧЕННОЙ ОТВЕТСТВЕННОСТЬЮ

УТВЕРЖДАЮ

Директор

ООО "РИЯ ФАРМАСЬЮТИКАЛ"

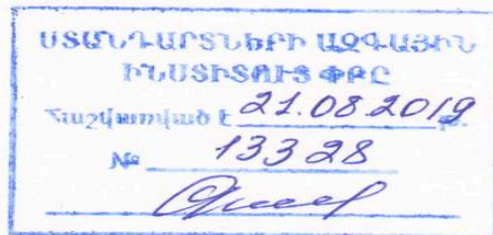


[Signature] Е.А.Мелконян

08.08. 2019 г.

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LIMITED LIABILITY COMPANY

APPROVED

By Y. A. Melkonyan,

Director

RHEA PHARMACEUTICAL LLC

/signature/

Seal

August 8, 2019

'Natural Guard'
FEED ADDITIVE
Specifications

TU AM 50106084.8409 -2019
Introduced for the first time

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2019

Natural oil blend formulation as an anti-African swine fever virus agent in *in vitro* primary porcine alveolar macrophage culture

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Abstract

Background and Aim: African swine fever is one of the severe pathogens of swine. It has a significant impact on production and economics. So far, there are no known remedies, such as vaccines or drugs, reported working successfully. In the present study, the natural oil blend formulation's (NOBF) efficacy was evaluated against ASFV *in vitro* using porcine alveolar macrophages (PAMs) cells of swine.

Materials and Methods: The capacity of NOBF against the ASFV was tested *in vitro*. The NOBF combines *Eucalyptus globulus*, *Pinus sylvestris*, and *Lavandula latifolia*. We used a 2-fold serial dilution to test the NOBF formulation dose, that is, 10⁵ HAD50/mL, against purified lethal dose of African swine in primary PAMs cells of swine. The PAM cells survival, real-time polymerase chain reaction (PCR) test, and hemadsorption (HAD) observation were performed to check the NOBF efficacy against ASFV.

Results: The *in vitro* trial results demonstrated that NOBF up to dilution 13 or 0.000625 mL deactivates the lethal dose 10⁵ HAD50 of ASFV. There was no HAD (Rosetta formation) up to dilution 12 or 0.00125 mL of NOBF. The Ct value obtained by running real-time PCR of the NOBF group at 96 h post-infection was the same as the initial value or lower (25), whereas the Ct value of positive controls increased several folds (17.84).

Conclusion: The *in vitro* trial demonstrated that NOBF could deactivate the ASFV. The NOBF has the potential to act as anti-ASFV agent in the field. The next step is to conduct *in vivo* level trial to determine its efficacy.

Keywords: African swine fever virus, *in vitro* trials, natural oil blend formulation, primary porcine alveolar macrophages cells.

Introduction

African swine fever virus (ASFV) reported as deadly for pigs. It is listed as a “notifiable disease” by the OIE due to high illness rates and a high mortality rate, up to 100%, and substantial financial losses [1-3]. Further spread of ASF to China has had disastrous consequences, especially instead of the fact that China contains more than half of the world’s pig population [4]. To date, as far as Vietnam is concerned, ASF has appeared in all 63 provinces of Vietnam, has destroyed more than 5.6 million pigs (more than 20% of total pigs), has decreased pork production by 8.3%, and has affected mainly small-scale farms [5-8].

The typical signs and symptoms of ASF are high fever, decreased appetite and weakness, difficulty

in standing, red or blue blotches on the skin (particularly around ears and snout), and, especially in sows, the symptoms of miscarriage, stillbirths, and weak litters can occur [9,10]. Like, diarrhea, vomiting, and difficulty breathing or coughing, the symptoms can also occur with the disease [9]. ASFV is a large, enveloped and structurally complex DNA virus with the *Asfarviridae* family’s icosahedral morphology. The virus can persist for a long time in the environment, carcasses, and various swine products. The vectors and carriers of the ASF virus are warthogs (*Phacochoerus africanus*), bush pigs (*Potamochoerus porcus* and *Potamochoerus larvatus*), and soft ticks (*Ornithodoros moubata*) [4] in which the virus is transmitted trans-steadily and through transovarial routes [9].

The role of natural oils as antiviral components is well known. As a standardized compound, natural products are significant components with antiviral properties [11]. A formulation was developed by blending three natural oils, *Eucalyptus globulus*, *Pinus sylvestris*, and *Lavandula latifolia*, with antiviral properties. Cineole, the significant component of eucalyptus oil, has potent anti-inflammatory and

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THỦ Y

Dịch tả heo châu Phi (ASF) và giải pháp ngăn chặn ở Việt Nam

ASF ĐÃ LÂY LAN NHANH VÀ XÂY RA Ở MỌI LOÀI HEO, MỌI LỬA TUỔI; VỚI TỶ LỆ CHẾT CÓ THỂ LÊN ĐẾN 100%, GÂY THIẾT HẠI KINH TẾ LỚN CHO NGƯỜI CHĂN NUÔI.

Nguyên nhân

Bệnh do virus *African Swine Fever* gây ra. Khi vào cơ thể heo, virus đi chuyển, tồn tại và phát triển ở mọi khi quan và mô bào của cơ thể. Chúng tồn tại và giữ nguyên độc lực tới 6 năm trong điều kiện lạnh, 4 - 5 tuần ở nhiệt độ phòng. Virus này có sức đề kháng rất mạnh với điều kiện tự nhiên. Bên cạnh đó, đường truyền lây ASF cũng rất đa dạng.

Triệu chứng

ASF biểu hiện ở 4 thể: Thể giả cấp tính, thể cấp tính, thể mãn tính và thể bệnh ẩn. Thời gian ủ bệnh của thể cấp tính từ 5 - 7 ngày, heo sốt cao 41 - 42°C, da đỏ hoặc tím, xuất hiện dịch rỉ mắt và chảy dịch mũi. Vật nuôi kém ăn hoặc bỏ ăn, đi lại khó khăn, khớp khằng, yếu ớt. Heo khó thở, chết đột ngột. Virus xâm nhập vào đường máu vì vậy gây nên những vết xuất huyết ở ngoài da, mắt. Nếu giải phẫu phát hiện được ruột bị xuất huyết; phổi, lách, thận bị sưng.

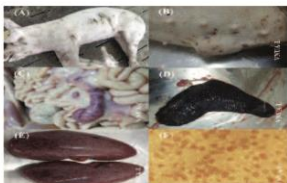
Nếu giải phẫu, phát hiện các hạch lympho sưng, xuất huyết; lách sưng, xuất huyết và nhu huyết; phổi sưng huyết, xuất huyết điểm, phù nề phổi và phế nang; gan và tử mật sưng, xuất huyết; thận xuất huyết điểm vô thận và xuất huyết ở nhu mô thận.

Giải pháp từ sản phẩm NATURAL GUARD

Khoa Thú y, Học viện Nông nghiệp Việt Nam kết hợp nhóm chuyên gia nghiên cứu của PT Asclepius Pharmaceutical Sciences Indonesia đang tiến hành nghiên cứu, thử nghiệm trên quy mô phòng thí nghiệm cho sản phẩm Naturalguard (NG) với đặc tính kháng virus gây bệnh ASF. Sản phẩm mở ra triển vọng lớn cho người chăn nuôi.

Phương pháp thí nghiệm

Các thử nghiệm *in vitro* và *in vivo* sử dụng sản phẩm NG đã được tiến hành để chứng minh khả năng kháng virus ASF. Các thử



Một số hình ảnh triệu chứng lâm sàng và bệnh tích đại thể của heo thí nghiệm (từ trái chung xuống) và tế bào PAMs (từ trái chung xuống *in vitro* gây nhiễm với virus dịch tả heo châu Phi (ASFV)). (A) Heo có triệu chứng hôn mê, co giật trước khi chết. (B) Xuất huyết điểm dưới da khu vực mắt, tai, ngực, bụng. (C) Hạch mang treo ruột sưng, xuất huyết. (D) Lách sưng, nhũ huyết. (F) Virus ASF nhàn lặn trên tế bào PAMs với các rosettes (đôi chung xuống *in vitro*)

ng nghiệm *in vitro* đã được thực hiện trên tế bào đại thực bào phổi heo (PAMs), và được coi là bước đầu tiên của thử nghiệm để xác định khả năng kháng virus ASF. Tế bào PAMs được phân lập từ heo khỏe mạnh am tính với các virus PCV2, FMDV, CSF, PRRSV và ASFV.

Kết quả nghiên cứu

Kết quả *in vitro* thông qua đánh giá HAD (Hemabsorption - Hấp phụ hồng cầu) hình thành các rosettes và realtime PCR cho thấy chủng virus ASF độc lực cao phân lập tại Việt Nam bị ức chế hay bất hoạt bởi sản phẩm NG ở các nồng độ pha loãng khác nhau. Sản phẩm NG dù ở nồng độ pha loãng cao 1/4096 vẫn có khả năng ức chế tốt virus ASF xâm nhiễm và nhân lên trên tế bào PAMs, không có tế bào nhiễm hay rosettes nào được phát hiện thông qua các vi trường soi kính hiển vi, trong khi đó ở nhóm đối chứng dùng virus ASF có khả năng xâm nhiễm và nhân lên mạnh với hiệu giá virus hơn



NATURAL GUARD: Improve Poultry, Swine and Cattle Health

Kết quả nghiên cứu cho thấy sản phẩm không độc cho heo và virus gây bệnh bị tiêu diệt hoàn toàn trong cơ thể heo sống khỏe mạnh và không phát hiện virus ASF trong suốt 2 tháng sau khi gây nhiễm.

10⁵HAD₅₀/mL. Kết quả realtime PCR đã chứng minh nhân dịch này, không có sự khác biệt về giá trị Ct giữa lượng virus sử dụng ban đầu (Ct = 25,12) và lượng virus ở các nồng độ khác nhau của sản phẩm NG ở các thời điểm 1, 2, 3 và 4 ngày gây nhiễm (giá trị trung bình Ct = 25,76), và khác biệt có ý nghĩa thống kê với nhóm đối chứng đương (Ct = 16,15) sử dụng cùng lượng virus ban đầu.

Đang chú ý, khi ủ virus ASF với liều 10⁵ HAD₅₀(liều gây chết 50% - lethal dose fifty, LD₅₀) với sản phẩm NG và gây nhiễm trên heo thí nghiệm bằng đường tiêm bắp góc tai cho kết quả tương đồng với kết quả thí nghiệm trên *in vitro*, heo khỏe mạnh, phát triển tốt và am tính với virus ASF trong suốt quá trình nghiên cứu theo dõi.

TS. TRƯƠNG QUANG LÂM¹, GS. TS. NGUYỄN THỊ LAN¹, THS. NGUYỄN THỊ HOÀ¹, PGS. TS. TÔ LÔNG THANH¹, TS. LÊ VĂN KHUÂN¹, THS. HUỖNG THỊ PHƯƠNG¹, TS. HAIG BABIKIAN² VÀ TS. RAJEEV KUMAR JHA²

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Research Article

EFFICACY OF NATURAL HERBAL FORMULATION “NATURALGUARD” AS AGP REPLACEMENT ON SWINE PRODUCTIVITY IN INDONESIA

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INTRODUCTION

Excessive utilization of Antibiotic and Growth Promoter (AGP) in animal farming stimulates the bacterial resistance. The AGP residue in animal products (meat, milk, and egg) is harmful to human health. The European Union has banned the utilization of AGP in animal farming since 2006. The government of Indonesia has banned the uses of AGP in livestock feed since 2018. The natural oils are one of the potential agents to replace the uses of antibiotics in the feed. It is a need of an hour to work on the development of a substitute for AGP in order to increase the productivity and to prevent various types of diseases. Essential oils are generally recognized as safe and recognized by the Food and Drug Administration (FDA). Essential oils can inhibit the growth of pathogen microbes in intestines and improve nutrient digestibility.

Natural guard (reg no Ty AM 50106084.8409-2019 in Armenia) is a feed additive containing three essential oils consisting of *Pine oil*, *Eucalyptus globules oil*, *Lavender oil*. The Natural guard acts as an immunomodulator that not only improves productivity but also expected to improve the quality of the pork. The present study was conducted to evaluate the efficacy of Natural guard as a potential agent to replace the AGP in swine feed.

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ABSTRACT

A study was conducted to determine the efficacy of the developed Natural Herbal Formulation Natural guard (reg no Ty AM 50106084.8409-2019 in Armenia) to replace the uses of antibiotics and growth promoters (AGP) in the feed. The objective is to improve the overall performance of swine including growth. Naturalguardcontains three natural essential oils, Pine oil, Eucalyptus oil and Lavender oil. Natural guard was mixed into the feed with 0,2% concentration. A total of 80 pigs in two groups were selected for the trial. Dietary of Natural Guard did not affect (P>0.05) the blood biochemical parameters. At the end of the experiment, Natural guard group swine gained 7 kg (12.28%) higher body weight compared to control. Feed intake did not differ (P>0.05) in both control and Natural guard groups. The feed efficiency data show ED that the dietary Natural Guard achieved lower FCR swine (P>0.05) 19.22% as compared to the control. The current trial outcome showed that the Natural guard has the potential to replace the uses of antibiotics and growth promoters (AGP) in swine feed.

MATERIALS AND METHODS

Experimental design and animals

A 90-day growth assay was performed to evaluate the efficacy of Natural guard on the productivity and blood parameters of the piglet. A total of 80 crossbred piglets (Yorkshire) i.e. 40 piglets of similar age, weight, and sex in each group, were assigned to two dietary treatments, control with Antibiotics and Growth promoter and without Natural guard supplement and treatment with Natural guard supplement (2 kg/ton feed) and without Antibiotics and Growth promoter. The experiment consisted of a randomized complete block design with two replicates of the dietary treatments.

The piglets were fed twice daily. Throughout the experiment, all piglets had *ad libitum* access to drinking water. Pigs were weighed on monthly (day 0, day 30, day 60 and day 90) basis and feed consumption was determined on a weekly basis. At the end of the experiment, blood samples were collected with a monojet standard hypodermic needle. Blood samples were collected in 3 ml tubes containing ethylenediaminetetraacetic acid (EDTA). Blood samples were used to study the blood biochemical and hematological parameters of experimental swine.

Novel formulation with essential oils as a potential agent to minimize African swine fever virus transmission in an *in vivo* trial in swine

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Abstract

Background and Aim: African swine fever (ASF) is currently the most prevalent disease in swine. The disease is spreading throughout primary swine-producing countries with heavy losses in population and revenue. To date, no successful vaccines or medications have been reported. This study aimed to design and develop a blend of natural essential oils and test its efficacy against the ASF virus (ASFV) in swine.

Materials and Methods: We attempted to develop a natural oil blend formulation (NOBF) and determine its efficacy against the ASFV. This study follows on from a previously published *in vitro* study that reported that the NOBF has anti-ASFV properties. A study was designed using 21 healthy piglets of triple-cross (Landrace + Yorkshire + Duroc) crossbred pathogen-free pigs with an average weight of 15 kg. The study consisted of NOBF-incubated, NOBF, positive control, and negative control groups. The NOBF groups were administered NOBF (80 mL/ton mixed in drinking water) beginning 10 days before the challenge and continuing throughout the experiment. The positive and negative control pigs consumed regular drinking water. The pigs were challenged by a sublethal dose of pure isolate ASFV strain Vietnam National University of Agriculture-ASFV-L01/HN/04/19 inoculation with 10^{3.5} HAD50/dose through the intramuscular route. There were six pigs in each group, three pigs directly IM challenged, and three pigs were considered cohoused pigs.

Results: Both challenged (three) and cohoused (three) pigs in the positive control showed clinical signs of ASFV infection, as detected by real-time polymerase chain reaction (RT-PCR) in blood samples, oral swabs, and feces. There was 100% cumulative mortality, that is, both challenged and contact pigs died in the positive control group on day 20 of infection. No signs of infection or mortality were observed in the NOBF-incubated group. The challenged pigs in the NOBF-direct challenge group showed clinical signs and mortality, whereas no clinical signs or symptoms occurred in the cohoused pigs. The immunoglobulin G (IgG) level of the contact pigs was the highest in the treatment group and the lowest in the positive control group. The IgM level of the contact pigs in the treatment groups was the lowest, whereas that of the positive control was the highest. The RT-PCR test showed that the ASFV was deactivated in the NOBF-incubated group. The challenged and contact pigs of the positive control group had high Ct values. The challenged pigs of the NOBF group had high Ct values, whereas the contact pigs from the same group and those of the negative control were negative for the ASFV, determined by PCR, in all samples. The comparison of the challenged groups showed that the appearance of the virus was delayed by at least 2 days in the NOBF group compared to the positive control group.

Conclusion: The results showed that NOBF can prevent the spread of the ASFV in a population. Moreover, NOBF can enhance the pig humoral immune system by enhancing IgG levels and reducing IgM levels. This study successfully demonstrated that NOBF is an anti-ASFV agent, which prevents horizontal transmission and enhances pig humoral immunity.

Keywords: African swine fever virus, *In vivo* trials, intramuscular challenge, natural oil blend formulation, Swine.

Introduction

The African swine fever (ASF) virus (ASFV) is deadly to pigs but harmless to humans [1]. ASF is one

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of the most severe viral diseases affecting pigs worldwide [2-5]. It is considered a “notifiable disease” by the Office International des Epizooties (International Office of Epizootics [OIE] of the World Health Organization because of its high mortality rate of up to 100% [6-8]. ASF causes acute hemorrhagic fever in domestic pigs and often results in significant economic losses because of the high rates of illness and death associated with the disease [8]. The introduction of ASFV into Denmark could result in losses of US\$12 million in direct costs and US\$349 million in



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MEEGID XV 15th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Disease – MEEGID XV (Online and On-Demand) Submission ID 29 Title Natural oil blend formulation (NOBF) protects Penaeus vannamei Boone, 1931 from white spot syndrome virus (WSSV) and enhances the productivity in the culture ponds Abstract White spot syndrome virus (WSSV) is lethal in penaeid shrimp. Successful efforts were made to develop a natural oil blend formulation (NOBF) with anti-WSSV properties using Eucalyptus globulus, Pinus sylvestris, and Lavandula latifolia in an equal proportion of water at a ratio of 1:1. A bioassay challenge trial was conducted using 1 g of 144 specific pathogen-free Penaeus vannamei Boone, 1931 samples in 4 aquarium replicates for each group. A NOBF dose of 0.2 ppm was applied throughout the trial period by mixing in aquarium water daily, starting seven days before challenge. The efficacy of NOBF against WSSV was measured using a modified per os method of challenge demonstrated in the current work. The cumulative mortality in the positive control group reached 89.6 % ten days post challenge. NOBF was also applied in six commercial shrimp ponds in a WSSV-prone area in East Java, Indonesia. Each pond was of equal size, 1000 m² and 1.2 m depth. The pathogenic Vibrio count of pond water was acceptable (<1000 cfu.mL⁻¹). The NOBF-applied ponds had better productivity (control 14.239 ton.ha⁻¹ and NOBF ponds 15.421 ton.ha⁻¹). The trial outcomes show that NOBF is safe and user-friendly, with properties that reduce WSSV load.

MEEGID XV 15th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Disease – MEEGID XV (Online and On-Demand) Submission ID 28 Title Identification of African Swine Fever Virus DNA polymerase X potential inhibitors in Natural oil blend formulation by Structure-Based Virtual Screening Approach. Abstract In this study, virtual screening approach was used to investigate Natural oil blend formulation (NOBF) individual compounds against African Swine Fever Virus (ASFV) DNA polymerase X (DNApolX). Using a combination of bioinformatics and computational tools, we predicted the interaction of dGTP binding pocket in the active site of ASFV DNApolX with 9 known constituted small molecules of blended essential oil formulation as well as natural ligand dGTP and known ASFV replication inhibitors roseterol and roseterolol as the reference compounds. We found that 91 docked small molecules interacted with common amino acid residues in the dGTP-binding pocket of ASFV DNApolX with high docking score and HF score value. hotspot residues of the enzyme (Fig. 4). Fig. Molecular interactions of reference molecules dGTP, oxyclohexanol and roseterol with ASFV DNApolX hotspot residues. Superimpositions of hotspot amino acid residues on the binding site structure-based model of the ASFV DNApolX and surface exposed amino acid residues are shown. Fig. 2D Interactions of dGTP with ASFV DNApolX. dGTP interacts with the hotspot residues of the PolX and forming van der Waals bonds with Val37 and Phe116 surface exposed hotspot amino acid residues. Unfavorable donor-donor and allylic interactions formed with Ser99 and Val120.

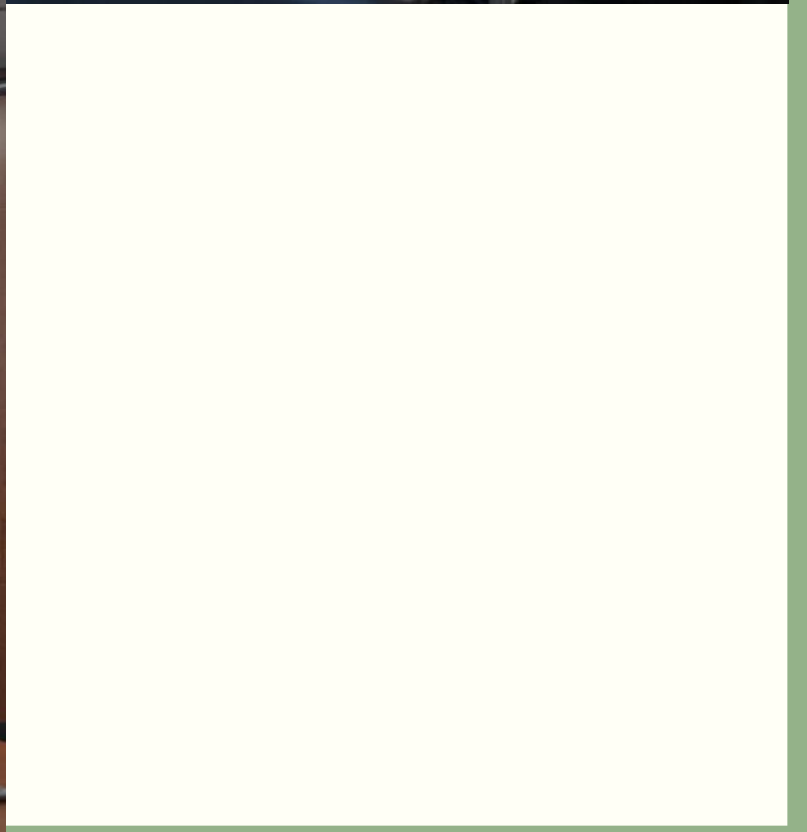
MEEGID XV 15th International Conference on Molecular Epidemiology and Evolutionary Genetics of Infectious Disease – MEEGID XV (Online and On-Demand) Submission ID 30 Title Development of essential oil blend formulation as a disinfectant against African Swine fever virus (ASFV) agent in PAM cells of Swine Abstract African swine fever virus has a significant impact on swine production and the economics of the swine-producing countries. The role of strict biosecurity measures becomes critical when there are no known remedies yet. Disinfection is considered a significant part of biosecurity measures and plays a vital role in reducing the risk of contaminating the environment. Successful efforts have been made to optimize a formulation Essential Oil Blend (EOB) product to determine its efficacy against the African swine fever virus (ASFV) in vitro conditions. The Essential Oil Blend (EOB) comprises a blend of, i.e., Eucalyptus oil, Gardenia oil, and Jasmine oil. The in vitro trial results demonstrated that EOB up to dilution ten could degenerate the lethal dose log 5 of ASFV. The work was shown by observing hemadsorption (Rosetta formation) and conducting a real-time PCR test. There was no Rosetta formation up to dilution 11 of EOB. The Ct value of the EOB group at 96 hours post-infection (hpi) was the same as the initial value or lower (25) than it, whereas the Ct value of positive control increased several folds (17.84). It is a potential water supplement to work against ASFV and enhance pig immunity to fight against common pathogens.

INTRODUCTION: AFRICAN SWINE FEVER (ASF) is caused by African Swine Fever Virus (ASFV). THE VIRAL GENOME contains 192 genes. IT WAS THE ASFV GENOME that was sequenced in 1970. THE MODE OF ACTION OF NOBF AGAINST ASFV IS UNKNOWN. RESULTS & DISCUSSION: Molecular interactions of reference molecules dGTP and roseterol with ASFV DNApolX hotspot residues. CONCLUSIONS: NOBF is safe and user-friendly, with properties that reduce WSSV load.

06. Virus Evolution 14:30 - 15:30. Chair: Martine Peeters. Attendees: Rajeev Kumar Jha, Takuya Kawahata, Mariana Kikuti, Maylis Layan, Marie Claire Morley, Alessandra Mozzi.

Posters: [P01] Comparative analysis of the protein expression in mosquitoes Aedes aegypti infected with ASFV. [P02] Identification of African Swine Fever Virus DNA polymerase X potential inhibitors in Natural oil blend formulation. [P03] Development of essential oil blend formulation as a disinfectant against African Swine fever virus (ASFV) agent in PAM cells of Swine. [P04] Subtyping of H. tuberculosis Beijing genotype indicates the absence of its transducer. [P05] Cryptosporidium spp. in Rattus rattus and Mus musculus domesticus from the Canary Islands. [P06] Molecular identification of Anisakis simplex s.s. and Anisakis paggalli in Scaber scotti. [P07] Presence of Cronosoma striatum in Anelasma algae of Tenerife, Canary Islands, Spain.

[P03] Development of Essential Oil Blend formulation (EOBF) as a Disinfectant against African Swine Fever Virus (ASFV) agent in PAM cells of Swine. Presenter: Rajeev Kumar Jha. INTRODUCTION: The African Swine Fever Virus (ASFV) is considered one of the most pathogenic viruses of pigs. Reducing the percentage of ASFV in the farm area is a vital point in controlling disease. The popular protein-rich disinfectants are not effective against ASFV as ASFV core is resistant to many disinfectants. The use of chemical disinfectants is also not recommended due to toxicity and residue retention. An essential oil blend formulation (EOBF) was developed using Eucalyptus oil, Gardenia oil, and Jasmine oil. The antimicrobial properties, efficacy, and compatibility were the main criteria in structuring the blend. Successful efforts were made to determine the efficacy of EOBF against ASFV in vitro conditions. The Essential Oil Blend (EOBF) comprises a blend of, i.e., Eucalyptus oil, Gardenia oil, and Jasmine oil. The in vitro trial results demonstrated that EOB up to dilution ten could degenerate the lethal dose log 5 of ASFV. The work was shown by observing hemadsorption (Rosetta formation) and conducting a real-time PCR test. There was no Rosetta formation up to dilution 11 of EOB. The Ct value of the EOB group at 96 hours post-infection (hpi) was the same as the initial value or lower (25) than it, whereas the Ct value of positive control increased several folds (17.84). It is a potential water supplement to work against ASFV and enhance pig immunity to fight against common pathogens. MATERIALS & METHODS: Efficiency of Essential Oil Blend Formulation (EOBF) against ASFV was demonstrated by in vitro conditions. The antimicrobial activity of EOBF was tested against ASFV in PAM cells of swine. Antiviral activity of EOBF in vitro was tested. CONCLUSION: The observed results demonstrated that EOBF up to dilution ten could degenerate the lethal dose log 5 of ASFV. The in vitro trial results demonstrated that EOB up to dilution ten could degenerate the lethal dose log 5 of ASFV. The work was shown by observing hemadsorption (Rosetta formation) and conducting a real-time PCR test. There was no Rosetta formation up to dilution 11 of EOB. The Ct value of the EOB group at 96 hours post-infection (hpi) was the same as the initial value or lower (25) than it, whereas the Ct value of positive control increased several folds (17.84). It is a potential water supplement to work against ASFV and enhance pig immunity to fight against common pathogens.







THANK YOU

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