

ROPADAIRY[®] FOR DAIRY COWS

Ropadairy[®] is oregano based natural feed additive which is used to improve general health, milk yield and reduce SCC score for dairy cows, manufactured by Ropapharm International BV under patent no EP 0828502.

Manufacturing story



Ropapharm International BV works with the Dutch horticulture research institute, PPO in Lelystad, to continue to improve the quality and consistency of the oregano plant. As a result of these studies Ropapharm International BV gain seeds from their own *Origanum onites* mother plants.

Plantapharm, cultivates which contains high amount of active component *Origanum onites* approximately 600 acres of plantation area at Antalya. *Origanum onites* also known as 'Turkish Oregano' in literature. Plantapharm obtains essential oil by steam distillation at the facilities which is located nearby plantation area. By its own plantation area and distillation facilities Plantapharm provides sustainable quality of raw material and control the amounts of active components in oregano oil which feed additives produced by Ropapharm International BV.

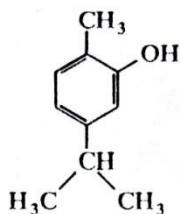


After obtaining essential oil of oregano process, Ropadairy[®] was manufactured under GMP and HACCP quality control management system, by Ropapharm International BV located in Zaandam, Netherlands. Ropadairy is available as powder, form for dairy cows.

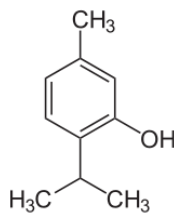
Composition of Oregano Oil

Essential oils are the secondary metabolites of plant materials that obtained by distillation. Essential oils are oily liquids consist of a lot of chemical components which can easily crystallized and have characteristic smell and flavor of obtained plant. Generally they are in liquid form, but named volatile oil because of their easily evaporation properties. These volatile components give plant a lot of properties such as antibacterial, antiseptic, anti-oxidative etc.

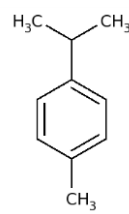
Oregano oil is the most known and used essential oil because of its strong antibacterial properties. The major active components of oregano oil are phenolic terpenoids such as carvacrol, thymol and p-cymene. These active components consist of great amount of oregano oil.



Carvacrol



Thymol



p-cymene

Oregano oil which is obtained from *Origanum onites* consist of 62-68 % carvacrol, 1-3 % thymol and 8 % p-cymene. Oregano oil shows biological effects at birds gastro-intestinal system because of these active components and with the synergism of other components exists in oregano oil.

In the year 2002, USA Agricultural Research Service (ARS) classified carvacrol as antibacterial, anti-inflammatory, antimelanomic, anti-oxidative, antispasmodic, carminative, flavor and thymol as antibacterial, anti-inflammatory, antimelanomic, anti-oxidative, antiseptic, carminative, flavor because of their biologic activities.

In our present day, carvacrol is used against infections and as anthelmintic. Also presents in medicines as a treatment factor against cold, flu, sore throat, ear ache, lung infections, infectious diseases, injuries, analgesics, arthritis, muscle spasms, headaches, E. coli, hepatitis, viral pneumonias, meningitis, Lyme disease, allergies, bronchitis, diarrhea, gingivitis, sinusitis, asthma, aphtha, eczema, neuritis, ankle twists, prostates, parasites, cricks, psoriasis, colitis, insects bite, gastritis and mycosis.

Also thymol used as anthelmintic, antiseptic present day and present in medicines against gingivitis.

Mode of action of oregano oil

Gastro intestinal system management is the most important issues to reach expected performance for animal husbandry. While microbial growth is desirable for digestion and increases performance, if the amount of pathogens increases, this will be effect negatively to the nutrient uptake and absorption and also performance too. Because of these reasons, antibiotics are used successfully as feed additives under the name of Antibiotic Growth Promoters (AGP) till EU ban. Because of residue and resistance problem related to public health issues using antibiotics as feed additives banned in EU countries and anticoccidials will be banned which is used highly amount at present day, in the year 2012.

At present day, preventive medicine is becoming more important issue because of public health and animal welfare. Consequently, Ropadairy® covers needs of dairy cows, because of active components that oregano oil includes.

Due to the active components of oregano oil, using Ropadairy® as a feed additive shows effects on dairy cows flavor, antibacterial, anti-parasitic, anti-oxidative, fungicidal, enzyme and immune stimulation.

Flavor properties of oregano oil

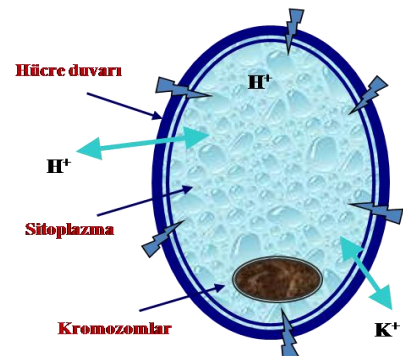
Monoterpenoids, which is made by two isoprene units, are main chemical structure of oregano oil. Isoprenoids are widely used in food industry as natural flavoring agent for a long time. These compounds stimulate scent and taste by effecting autonomic and central nervous system. By triggering of these sensations, motility of intestines will increase and endocrine system will be stimulated to produce digestive enzymes.

Antibacterial properties of oregano oil :

Oregano oil shows antibacterial effect by courtesy of carvacrol, thymol and p-cymene. These major compounds of oregano oil are monoterpenoid phenols belong to hydrocarbon class.

Terpenoids such as thymol and carvacrol, shows their antibacterial effects through interaction with cell membrane of microorganisms. Because of hydrophobic properties of these compounds, they can easily diffuse lipid layer of cell membrane and cause pores between fatty acids. These conformational alterations change permeability of cell membrane for cations H^+ and K^+ . Bacteria death will occur because of inhibition of essential processes such as ATP synthesis due to ion concentration alteration and cytoplasm loss.

Antibacterial properties of oregano oil have bactericidal effect both for gram positive and negative bacteria, because of hydrophobic character of active components of oregano oil. The differences between gram positive and negative bacteria don't change bactericidal effectiveness of oregano oil. The cell wall of gram negative bacteria which has hydrophilic properties, do not



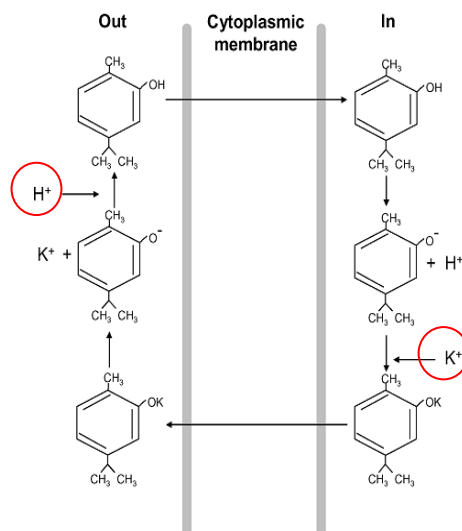
ROPADAIRY® FOR DAIRY COWS

permit passing through inside of lipophilic substances such as monensin and active compounds of many essential oils. But hydrophobic substances such as thymol and carvacrol which has low molecule mass can diffuse inner side of cell wall by making hydrogen bonds with cell wall and show permeability change effects on cell membrane.

In the year 2002, Ultee shows different mode of action for carvacrol. He shows that hydroxyl substances of phenolic compounds carry ions and protons in and out from cell membrane just like ionophore antibiotics mode of action. This mode of action related to hydroxyl substances viable only for of aromatic compounds. Because hydroxyl substances of menthol which has exactly same chemical structure with carvacrol doesn't shows same mode of action for ions and protons.

Bacterial resistance is not an issue for oregano oil because of this different mode of action of oregano oil. Oregano oil is the most well known and used between essential oils because of strong antibacterial properties.

Also synergism has been observed between carvacrol and its precursor p-cymene in many studies. Synergic and additive effects between major and minor compounds of oregano oil became more potent to the microorganisms. Also you can observe the results of study which is made by Ultee (2000) about the time of bacteria death presence of carvacrol and p-cymene alone or both at the table given below.



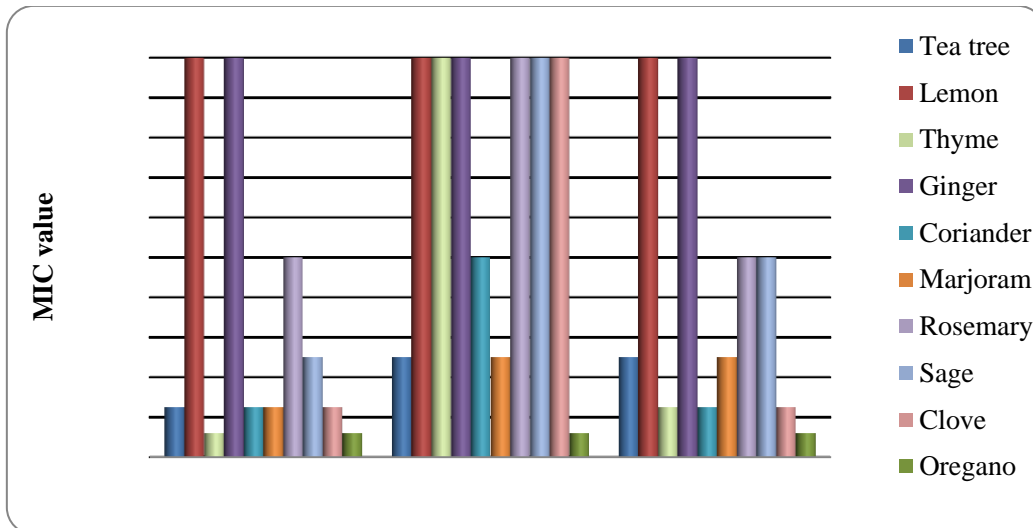
Carvacrol (mM)	Cymene (mM)	Carvacrol + Cymene (mM)	Time needs to kill 50 % of bacteria population (t0.5)
1.0	-	1.0	>100
2.0	-	2.0	3
-	0.5	0.5	>100
-	6.0	6.0	3
Combinations			
0.5	0.25	0.75	48
0.5	0.5	1.0	9
1.0	1.0	2.0	>1

The summary of some studies defining MIC (Minimum Inhibition Concentration) ($\mu\text{l ml}^{-1}$) value interval of carvacrol and thymol on important pathogens for poultry is given below.

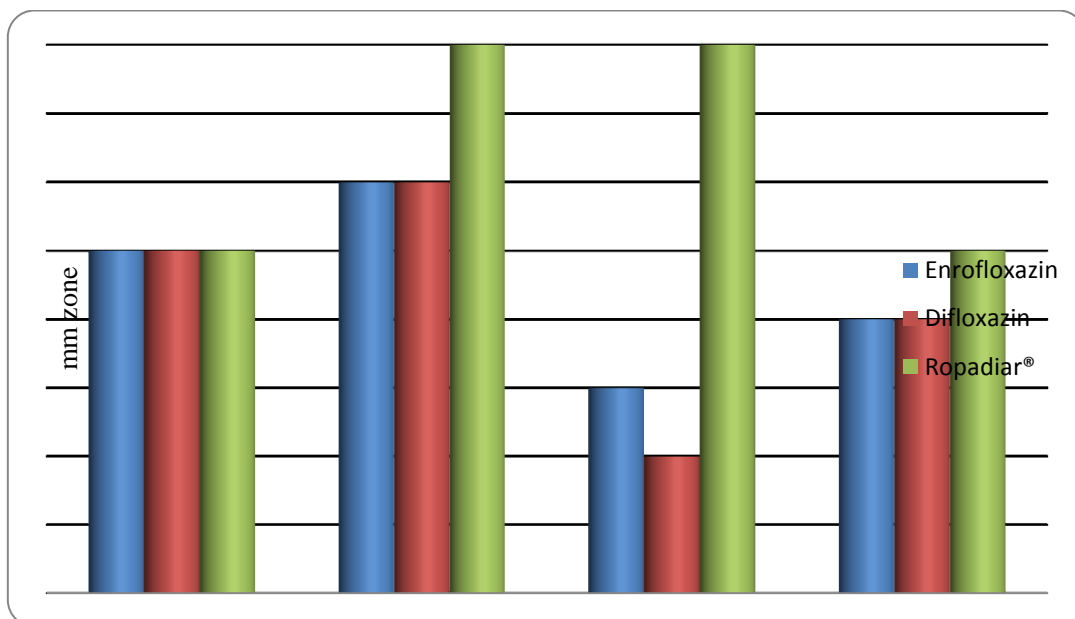
	E. coli	S. typhimurium	S. aureus
Carvacrol	0,225 - 5	0,225 - 0,25	0,175 - 0,450
Thymol	0,225 - 0,45	0,056	0,140 - 0,225

ROPADAIRY® FOR DAIRY COWS

You can observe the results of study about in vitro antibacterial effects of some essential oils which is made by Hammer (1999).

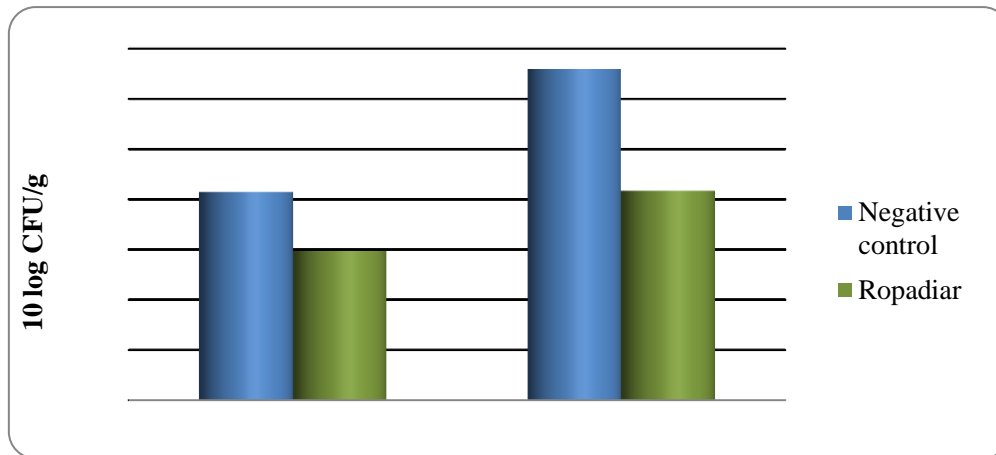


This study was designed by German Poultry Federation Clinic to observe the effects of different antibiotics and Ropadiar® on *E. coli*, *S. typhimurium var. Copenhagen* and two different *Staphylococcus* serotype which was isolated from field. You can see the inhibition zones of 5 mg of Enrofloxazin, 10 mg of Difloxazin ve 5 mg of Ropadiar® at the chart which is given below.



ROPADAIRY® FOR DAIRY COWS

This study was designed to observe the presence of *Cl. perfringens* at 10,000 broilers ileum and cecum which are fed with normal diet and added Ropadiar® in the year 2000 at Japan. You can see the results at the chart given below.



You can see the results of antibacterial effectiveness of oregano oil on microorganisms at the table given below.

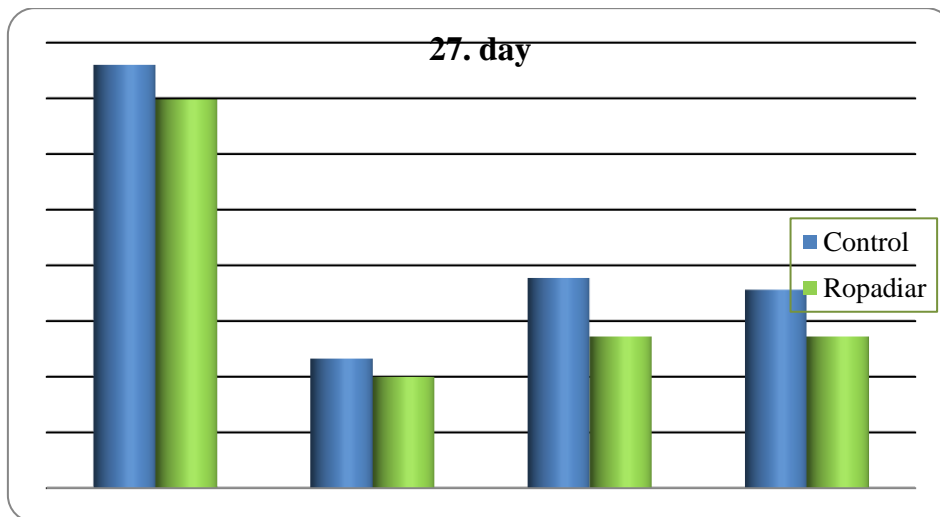
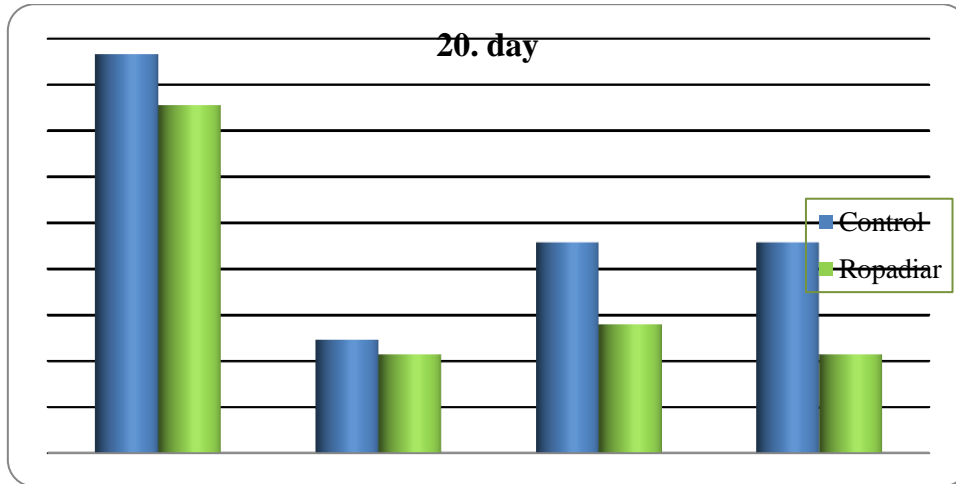
Antibiogram studies related Oregano oil							
Staphylococcus aureus	+++	Enterobacter aerogenes	+++	Klebsiella pneumoniae	+++	Mycobacterium tuberculosis	++++
Escherichia coli	++++	Streptococcus uberis	+++	Corynebacterium pyogenes	+++	Aspergillus spp.	++++
Pasteurella multocida	++++	Proteus rettgeri	+++	Pseudomonas aeruginosa	+++	Mucor spp.	+++
Streptococcus faecalis	+++	Salmonella spp.	++++	Cryptosporidia spp.	++++	Eimeria spp.	++++
Streptococcus agalactiae	+++	Proteus vulgaris	+++	Streptococcus pyogenes anim.C	+++	Candida spp.	+++
Vibrio coli	++++	Proteus mirabilis	+++	Erysipelothrix insidiosa	+++	Treponema hyodisaenteriae	++++

Anti-parasitic properties of oregano oil :

Anti-parasitic properties of oregano caused by phenolic compounds of oil. These active compounds speed up the process of the normal shedding of enterocytes from the intestinal mucosa. Normally, enterocytes which are found in the first layer of intestinal mucosa shed between 4 and 7 days. The presence of active components of oregano oil intracellular coccidia sporozoites are shed before they have the opportunity to penetrate deeper into the gut wall or accomplish their life cycle and form new oocysts.

ROPADAIRY® FOR DAIRY COWS

You can see Ropadiar® effects on lesions and oocyte output at the 20th and 27th day under mild coccidiosis infection. Contrary to control group, Ropadiar® group was given 0,1 ml per metric ton of drinking water.



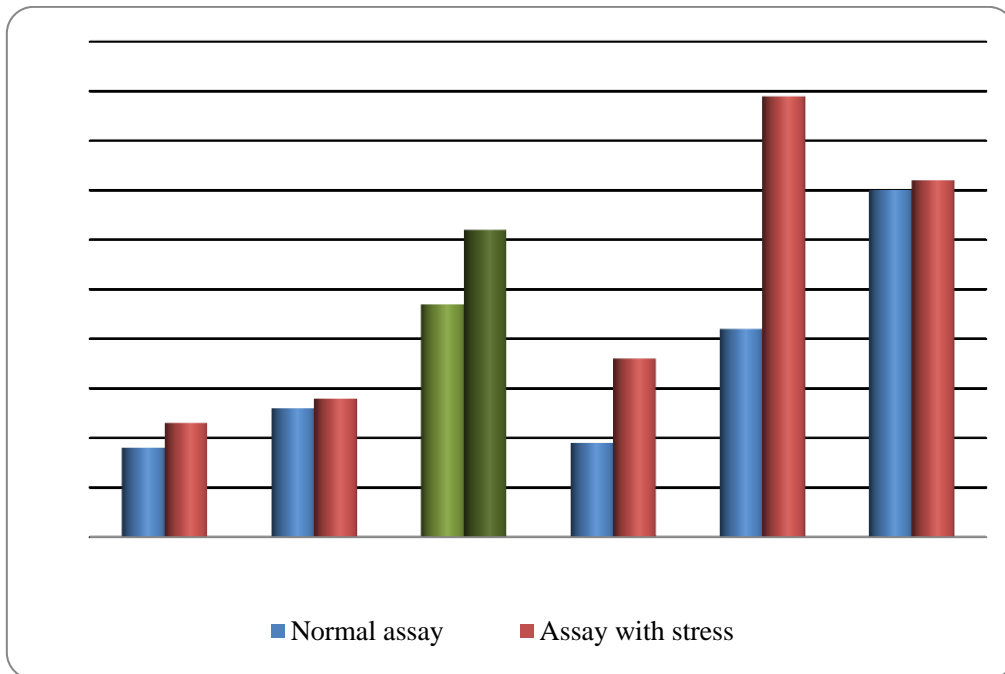
Redmann designed a study at Gissen University to observe effects on clinical symptoms and mortality of using two different dosage of Ropadiar® after clinical symptoms have seen on turkey Blackhead disease.

Trial	Ropadiar® dosage	Clinical symptoms end (day)	Mortality ended due to histomoniasis (day)
1	4 gr/kg	3	2
2	3 gr/kg	3	2

Anti-oxidative properties of oregano oil:

The active compounds of oregano oil have strong anti-oxidative properties. Anti-oxidative mode of action of oregano oil will both protect feed material while storage and also in the digestive track from oxidation. By the same time, the active compounds of oregano oil will protect intestine villies from peroxidation by reducing free radicals which are occurred phagocytosis action of macrophages. Consequently, by this bilateral protection absorption of nutrients will increase.

This study was designed by Deans (1998), to observe some essential oils anti-oxidative index and compare with alfa-tocopherol (vitamin E) and Butil Hidroksi Toluen (BHT) which are mostly used in practice.



Govaris (2005) showed that, dietary oregano reduce rate of malondialdehyde (MDA) which is occurred as a result of oxidation at storage and also reduce total bacteria load and *Pseudomonas spp.* storage at refrigerator conditions (+4°C) at turkey breast meat.

Fungicidal properties of oregano oil :

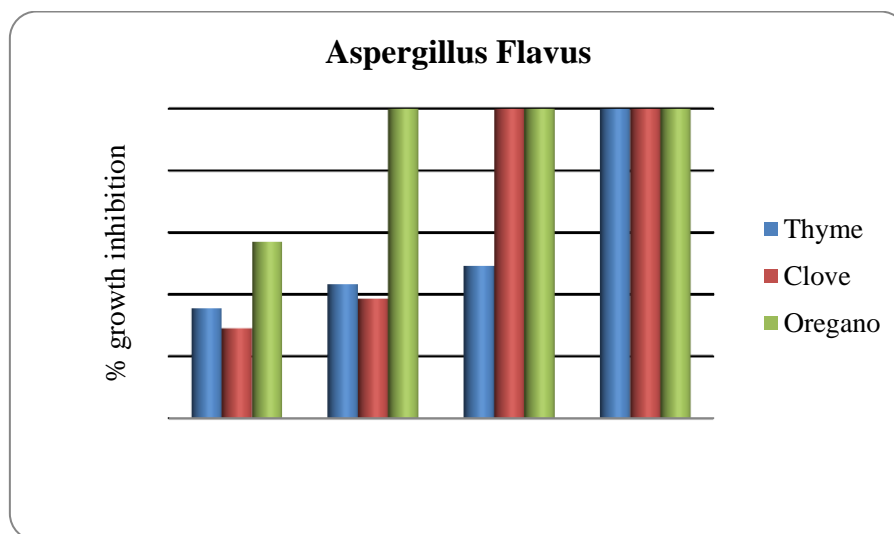
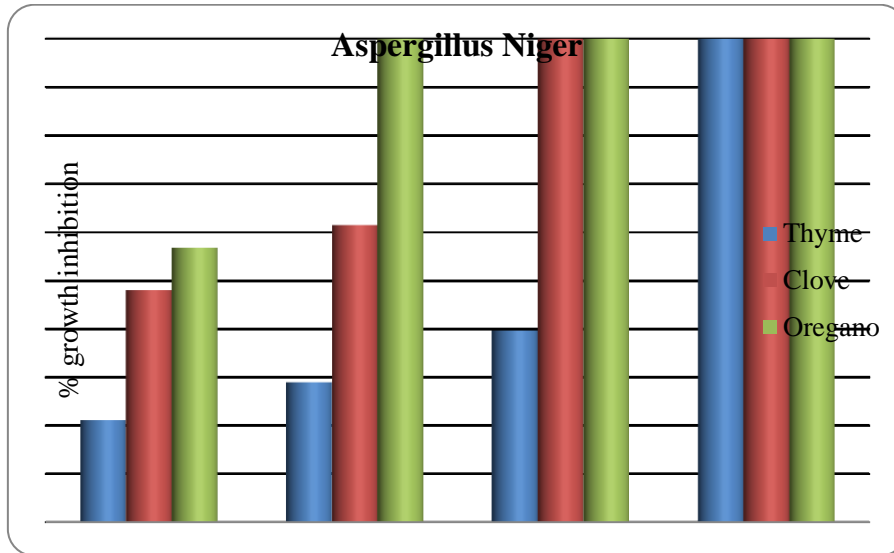
Oregano oil has fungicidal effect by phenolic compounds such as carvacrol and thymol in its chemical structure. Because of this mode of action using oregano oil as a feed additive will protect feed material from yeast and mold at storage conditions.

Fungicidal effect of oregano oil is related to its chemical composition. Biondi (1993) show that, *O. onites* essential oil which is rich from carvacrol, has more fungicidal effect on *A. niger*, *A. terreus*, *C. albicans* and *Fusarium spp.* than other oregano species.

ROPADAIRY® FOR DAIRY COWS

Fungicidal effects of oregano oil on *A. niger* and *A. flavus* which are responsible from food spoilage and/or food borne disease, is important for food and feed safety issues.

You can observe the fungicidal effects of different concentrations of some essential oils inhibition percentages of growth on *A. niger* and *A. flavus*.



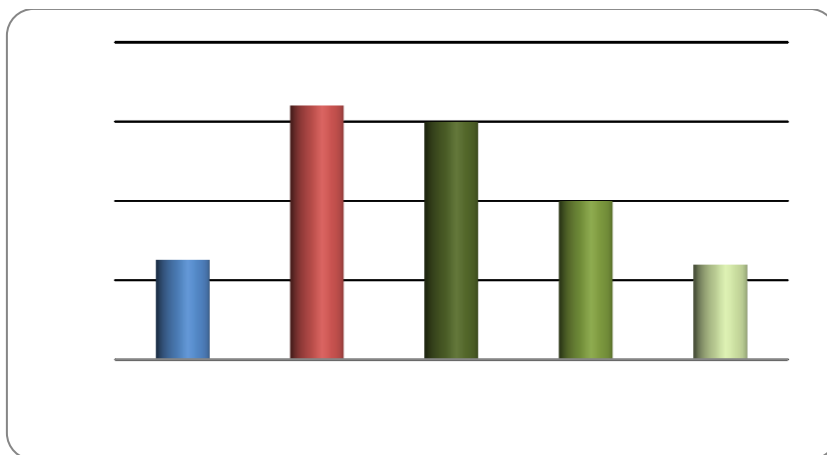
Immune stimulation properties of oregano oil :

Health management and biosecurity, became most critical issues for animal breeding. It is complicate to provide ideal isolation from pathogens at intensive poultry breeding conditions. Therefore, developing optimum immune response is the main issue for breeding. Although, young animals are under exposure of pathogens, there will be no change at performance if the immunity is adequate.

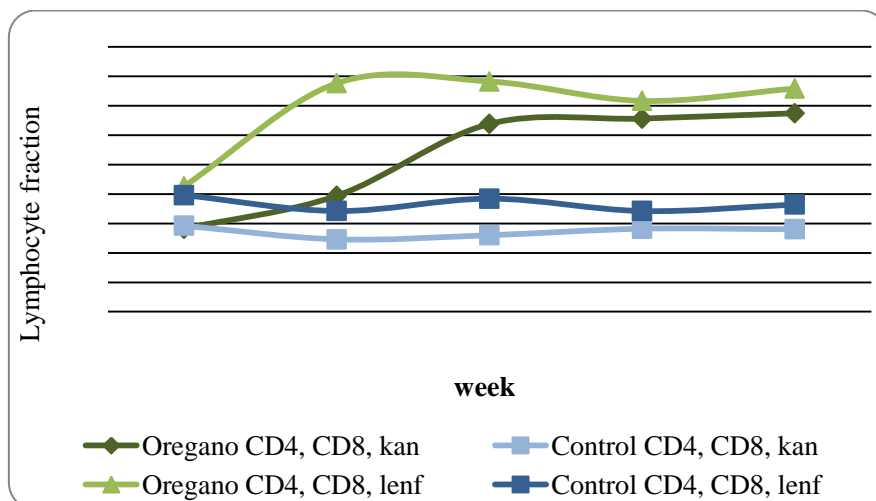
ROPADAIRY® FOR DAIRY COWS

The studies show that, oregano oil has in vitro antiviral effect and immun stimulation effect both in vitro and in vivo. Using oregano oil as feed additive will increase humoral immune response.

This study was designed at Wageningen University (2004, Netherlands) to show immune response of oregano. You can observe immune stimulation effect of Ropadiar® in carp head kidney cells.



This study was designed by Walter (2004), to show effects of oregano oil on humoral immunity (CD4, CD8). You can observe the results at the chart given below.



ROPADAIRY® FOR DAIRY COWS

Product info



Ropadairy®

Ropadairy®, provides continuity of healthy digestive system for cows and also reduce weaning stress at calves because of active compounds of oregano oil. Regular use of Ropadairy® will increase milk yield and reduce SCC score which is an indicator of mastitis.

Ropadairy® contains %5 of high quality oregano oil obtained from *Origanum onites*, which are specially selected and growth under optimal conditions. Ropadairy® contains wheat flour as a carrier. Ropadairy® can be storage for 2 years in its original packaging in a cool and dry place.

Recommended dosage for Ropadairy®

Per cow

5-7 gr/day



Ropaject®

Ropaject® which is an emulsifier of sunflower oil and oregano oil is used for treatment of calf's diarrhea applied by orally.

Ropaject® effects in a very short time and reduces losses minimum due to diarrhea. Only one application of Ropaject® after defecation of meconium will be enough for prevention of diarrhea at calves.

ROPADAIRY® FOR DAIRY COWS

Studies with Ropadairy®

Ropadairy® rumen effect

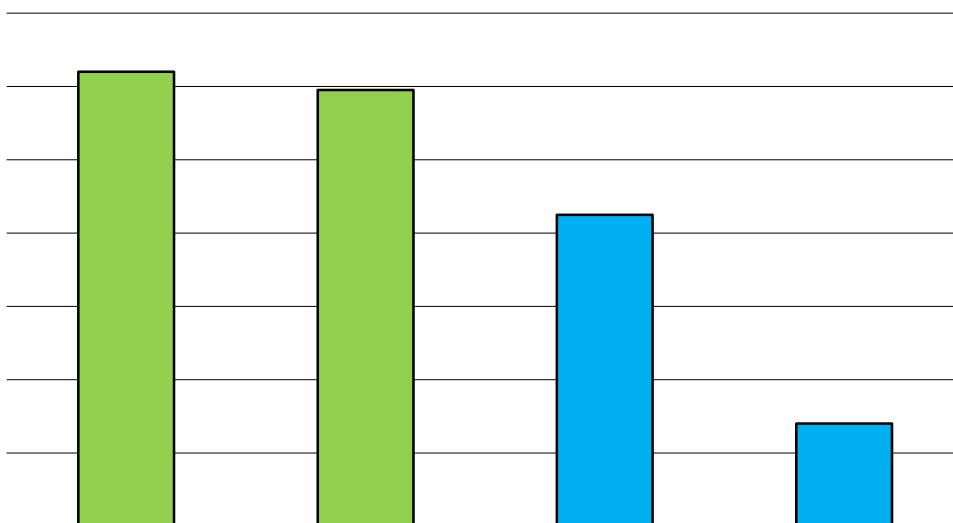
This study was designed by FIS (Feed Innovation Service) in the year 2006 to observe in vitro effects of Ropadairy® on rumen volatile fatty acids. You can see the results at the table given below.

	Control	Ropadairy (High dosage)	Ropadairy (Low dosage)
Total volatile fatty acid (mmol/g)	4.73 a	5.08 b	4.78 a
Acetic acid (mmol/g)	2.79 a	3.15 b	2.87 a
Propionic acid (mmol/g)	1.07 a	1.20 b	1.11 a
Methane (%)	9.28 b	8.65 a	8.91 ab

Ropadairy® effects on milk yield

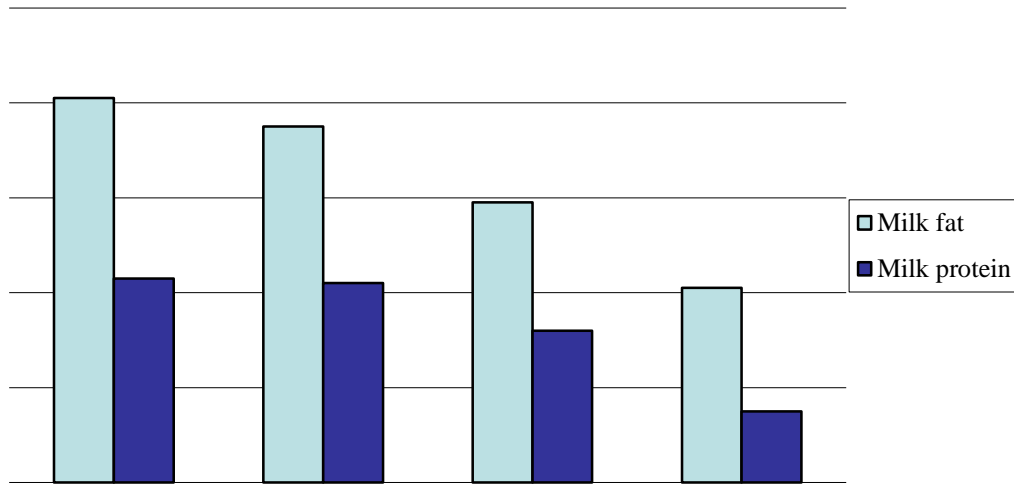
De Boer and Hoekstra designed two studies to show effects of Ropadairy® on milk yield. In the first study, they observe milk yield quantity of 95 milking cow which are given 8 gr of Ropadairy® daily for the first 6 weeks and last 6 weeks without additive.

You can observe the results of Ropadairy® effect on milk yield at the chart given below.

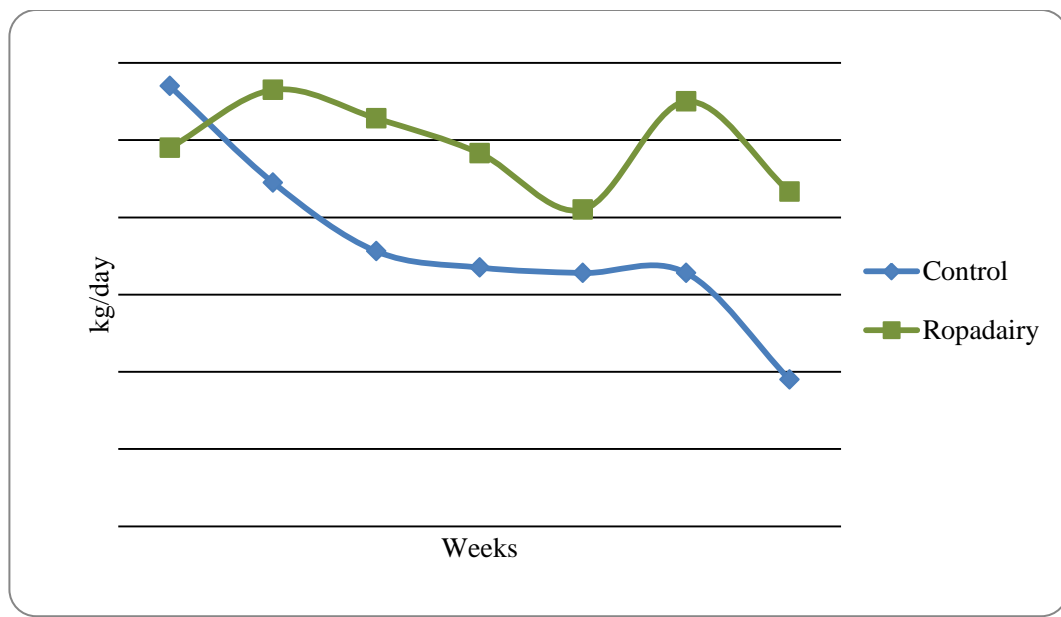


ROPADAIRY® FOR DAIRY COWS

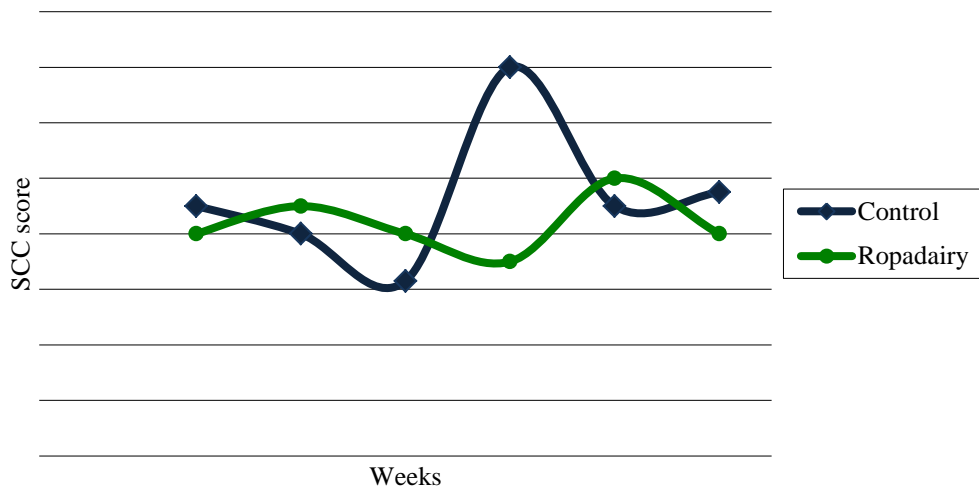
You can observe the results of Ropadairy® effect on milk protein and fat at the chart given below.



On the second study De Boer and Hoekstra chose 20 milking cow which are identical milk yield properties and same period of lactation. They designed 2 groups; one group is given 8 gr of Ropadairy® for 12 weeks and the other group designed as control group. You can observe the differences between groups on milk yield and SCC score at the charts given below.



ROPADAIRY® FOR DAIRY COWS



This study designed by Smitzis in the year 2007 to show Ropadairy® effects on sheeps. The study continues for three months on 60 sheep. You can see the figures for milk yield, fat percentages, protein percentages, lactose percentages and SCC scores at control and Ropadairy® groups.

	Control	Ropadairy®
Milk yield (ml/day)	924	966
Fat percentage	5.81	6.02
Protein percentage	5.42	5.75*
Lactose percentage	4.68	4.70
SCC score (x10³)	1575	1272

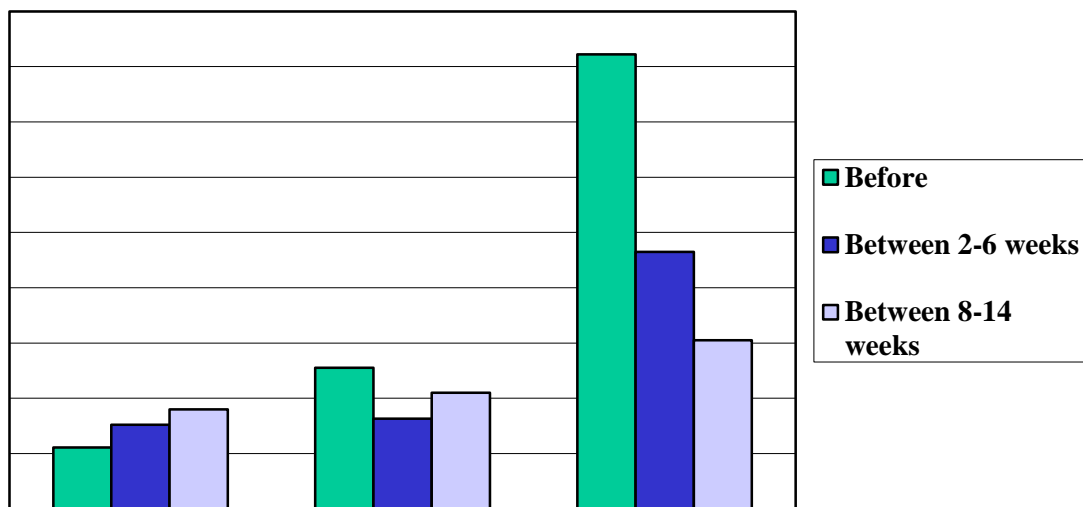
Dankers made a field trial with 150 milking cow in Germany at 2009, in this trial he showed that giving 7 gr of Ropadairy® daily increases milk yield within 3 weeks from 27 kg to 29.2 kg without any changes for milk protein and fat percentages.

Also Plantapharm, made a field trial with 50 milking cow in Turkey at 2009, in this trial we showed that giving 7 gr of Ropadairy® daily increases milk yield from 20.12 kg to 22.02 kg within one week.

ROPADAIRY® FOR DAIRY COWS

Ropadairy® effect on SCC score

This study designed on 170 milking cow in South Korean at 2007 to show effects of Ropadairy® on SCC score. Ropadairy® was given daily amount of 1 gr to kg of compound feed. Cows are divided into three groups by their SCC score which is lower than 200.000 (%49), between 200.000 and 400.000 (%24) and over than 400.000 (%27). You can observe effects of feeding with Ropadairy® for 14 weeks on SCC score at the chart given below.



Studies with Ropaject®

This study was under taken in Italy to show effects of Ropaject® on calf's diarrhea. Ropaject® was applied one times orally and applied for second times if it's necessary. For antibiotic group three different antibiotics used for different calves. Administration dosages for antibiotics are like this, Baytril %2,5 5 mg/day for 3 days orally, Bacolam (amoxicillin-colistin) 10ml/100 kg for 3 days and Biosol (neomicin sulfat) 18 mg/kg for 5 days. You can see the results at the table given below.

	Ropaject® group	Antibiotic group
Number of calves	43	11
Success treatment with one application	25 (%58)	
Success treatment with second application	17 (%40)	
Number of treated calves number at the end	42* (%100)	11 %100)

*For one calf applied third dosage on the 7th day.

ROPADAIRY® FOR DAIRY COWS

Colibacillosis

625 calves 3-5 days old suffers from diarrhea characterized with bad smell, yellow-darkish strong diarrhea and no appetite. 381 of calves are treated with Ropaject® and 244 of them treated with Enterotonon (recommended dosages). You can observe the number of treated calves and time to need for treatment at the table given below

Time after first application (hours)	Treatment rate with Ropaject® (%)	Treatment rate with Enterotonon (%)
8	69	
12		16
16	82	
24	98	23
32	100	
36		33
48		48
60		54
72		69
84		75
96		83
108		93
120		97
144		100
Number of death	2 (%0,52)	12(%4,92)

Salmonellosis

416 calves 5-10 days old suffers from diarrhea characterized with bad smell, white strong diarrhea and no appetite. 231 of calves are treated with Ropaject® and 185 of them treated with Baytril (recommended dosages). You can observe the number of treated calves and time to need for treatment at the table given below.

Time after first application (hours)	Treatment rate with Ropaject® (%)	Treatment rate with Baytril (%)
8	32	
16	54	
24	72	
32	83	
40	94	
48	97	11
56	100	
72		25
96		50
Number of death	4 (%1,73)	18 (%9,72)
		After 96 hour treatment stopped and treated with Ropaject®

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Acute catarrhal enteritis

125 calves suffer from common diarrhea caused by different bacteria. 71 of calves are treated with Ropaject® and 54 of them treated with Enterotonon (recommended dosages). You can observe the number of treated calves and time to need for treatment at the table given below.

Time after first application (hours)	Treatment rate with Ropaject® (%)	Treatment rate with Enterotonon (%)
8	83	
16	100	
72		100
Number of death	0	0

Ropaject® and Ropadairy® effects on neonatal calf diarrhea

For this purpose, Ropaject® applied to 294 of calves after defecation of meconium on the second day. Ropadairy® was given for 45 days by mixing milk on third day amount of 1 gr per 1 liter of milk. Control group which is 285 calves given nothing. You can observe diarrhea rate of groups during trial period at the table given below.

Time after first application (days)	Diarrhea % of Ropaject® group	Diarrhea % of control group
1	1,02	4,91
2	1,36	8,07
3	2,04	14,03
4	3,40	17,19
5	2,04	21,05
6	3,40	22,81
7	2,38	17,89
14	2,72	25,96
21	2,04	Observation of control group
28	3,40	stopped and calves treated
35	2,72	with Ropaject®
42		
45		
Number of death	3 (%1,02)	12 (%4,21)

ROPADAIRY[®] **FOR DAIRY COWS**

Philosophy of Ropadairy[®]

Benefits of Ropadairy[®] using as a feed additive not only considered as increasing milk yield and reducing SCC score but also put in improving general health and provides extra protection for stressful periods.

Also protect feed material during storage and increase feed quality by fungicidal and anti-oxidative properties of active compounds of oregano oil.

Ropadairy[®] eliminates pathogens in digestive system and provides protection for diarrhea cases. Increase nutrient absorption by protecting intestinal villies. Reduce performance loss caused by stressful periods. Also improve herd health by increasing immunity.

Ropadairy[®] provides measurable yield increase, and also helps sustainability of general herd health.