Your way
to success

KOKCISAN® 120G

12% granulate

salinomycin sodium

Broad-spectrum anticoccidial for poultry

TECHNICAL MANUAL

KRKA

CONTENTS

1 ▸ KOKCISAN IS PRODUCED IN ACCORDANCE WITH THE GMP, FDA AND ISO 9001 AND 14001, OHASAS (18001) AND HACCP STANDARDS

Kokcisan is produced by a conventional fermentation process using a highly selected strain of the micro-organism *Streptomyces albus* forming typical white colonies.
For the fermentation process, vegetable oil, soybean flour, corn starch, calcium carbonate and different minerals are used.
On completion of the fermentation the broth is treated with sodium hydroxide (to convert the salinomycin molecule to the more stable sodium salt) and inert carriers (e.g. calcium carbonate) are added.

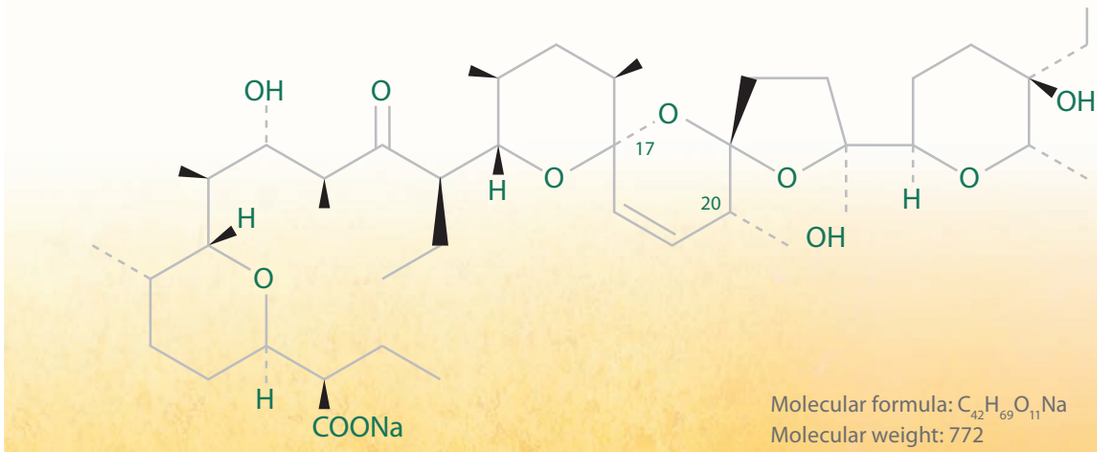
Micro-organism Streptomyces albus forming typical white colonies

Treated fermentation broth is spray dried. The obtained salinomycin sodium concentrate represents the starting material for the final Kokcisan formulation, using excipients, such as calcium carbonate. Granulation is performed on dedicated line, using the wet granulation principle with sucrose as the binder. The granulate is then dried and sieved; only the specified particle size range is collected.
The whole production process is automatised and utilises the most advanced technology complying with the GMP, ISO, FDA and HACCP directions and rules.

2 ▸ SALINOMYCIN IS AN ANTIBIOTIC AND COCCIDIOSTAT FROM THE IONOPHORE FAMILY, WHICH IS NOT USED IN HUMAN MEDICINE OR VETERINARY THERAPY AND THE CHEMICAL FORMULA OF WHICH BEARS NO SIMILARITY TO THOSE OF OTHER ANTIBIOTICS MOLECULES CURRENTLY IN USE

Salinomycin is an ionophore antibiotic with a pseudocyclic structure characterised by infolded oxygen atoms allowing cation binding; the nonpolar outer section ensures solubility in the lipid membrane.

Structural formula of salinomycin sodium



Molecular formula: $C_{42}H_{69}O_{11}Na$
Molecular weight: 772

MECHANISM OF ACTION

The activity of salinomycin is similar to that of other ionophore polyether antibiotics.

ANTIBACTERIAL ACTIVITY

Salinomycin is an antibiotic and coccidiostat from the ionophore family, which is not used in human medicine or veterinary therapy and the chemical formula of which bears no similarity to those of other antibiotics molecules currently in use. Its sphere of activity essentially covers gram-positive bacteria and anaerobic bacteria (*Clostridium perfringens*). The molecule has no cross resistance with other antibiotic molecules or adverse effects on transfer or on the excretion of certain zoonotic bacteria, nor does it induce resistance to the molecule in certain intestinal bacteria.

ANTICOCCIDIAL ACTIVITY

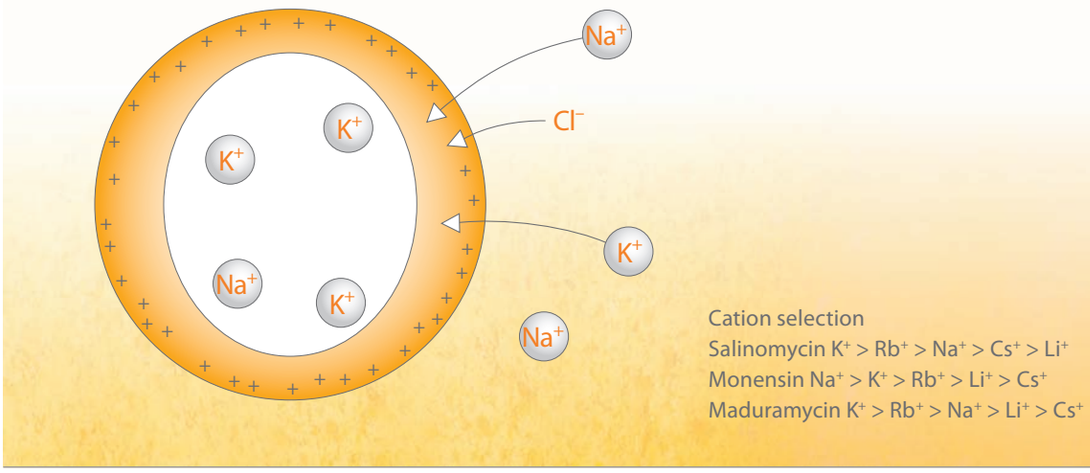
The anticoccidial activity of salinomycin against the *Eimeria* species which infect the intestine of chickens is well established and has been the subject of numerous publications since the substance was first synthesised in the year 1969.

Salinomycin is effective at an early stage of coccidial life cycle in the intestine of chickens, and exerts its anticoccidial action by preventing the introduction of sodium and potassium ions into the metabolic processes of the protozoal cells during the schizogenous phase. This action causes disruption to the intracellular ion concentration in coccidia, thus reducing the number of oocysts.

Salinomycin penetrates across the membrane of the coccidial cell and causes a change in the permeability of the lipid barrier by disrupting ion transition inside and outside of the cell membrane of the parasite.

Salinomycin is a potent transmitter of K^+, Rb^+ and Na^+ from the cellular mitochondria. It has been shown to be a weak transmitter of Cs^+ and Li^+.

Penetration across the lipid barrier



COCCIDIOSIS IN BROILERS

Coccidiosis remains one of the most important diseases in commercial poultry production. It is a disease caused by protozoan parasites which invade and multiply within the epithelial cells lining the intestine. In chickens, these parasites are from the *Eimeria* genera. Seven *Eimeria* species are recognised to affect broiler chickens: *E. acervulina*, *E. maxima*, *E. tenella*, *E. brunetti*, *E. necatrix*, *E. mitis* and *E. praecox*.
All species of coccidia are pathogenic, although some of them show a lesser degree of pathogenicity than others. The highest mortality is associated with *E. tenella* and *E. necatrix* infections in broiler chickens. Infections due to the other species of coccidia result in reduced weight gain, anorexia, dehydration, secondary infections, reduced absorption of nutrients, and they may also enhance the occurrence of other diseases.

The incidence of coccidiosis does, however, not depend solely on the number of undigested oocysts. There exist several other important factors linked to the production technology, which may have an important impact on the incidence of the disease. These include population density, the size of the broiler farm, litter quality, zoohygienic conditions and the previous use of coccidiostats. Other factors that may have an impact on the occurrence of the disease include lighting schedule, breed type, biosecurity, other viral and bacterial diseases, the presence of mycotoxins in feedingstuffs, and locomotor diseases.

For a reliable clinical diagnosis of coccidiosis under conditions of intensive rearing of broiler chickens, close attention should be paid not only to the parasitological examination for detection of oocysts in feces, litter and intestinal wall, but also to the localisation and characteristic features of intestinal lesions.

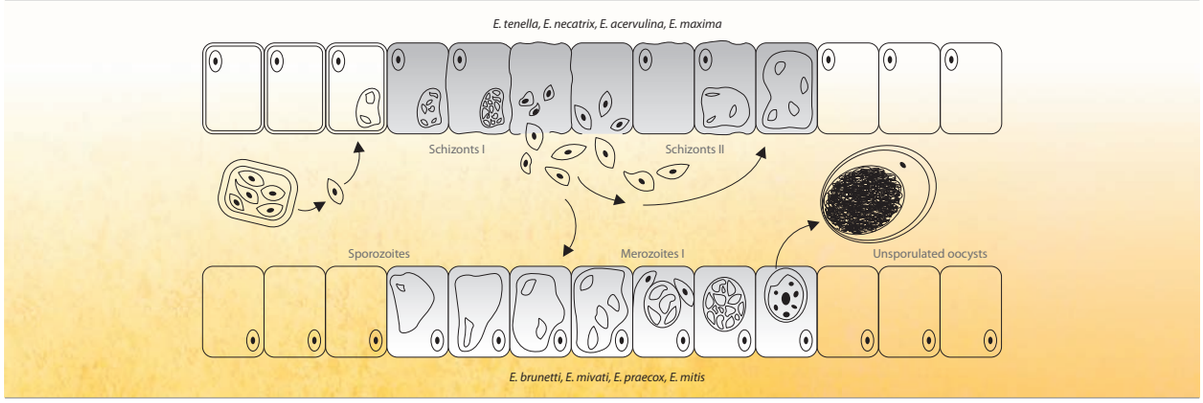
Typical macroscopic lesions seen in a coccidial invasion

Invasion site	Macroscopic lesions	Coccidia
Upper duodenum	Small, white, circular patches	*E. mitis*
	Large elongate patches	*E. acervulina*
Central portion of the small intestine	Petechiae and intestinal swelling	*E. maxima*
	Enlarged white patches and bloody intestine	*E. necatrix*
Distal portion of the intestine	Petechial bleeding	*E. necatrix*
	Necroses and intestinal inflammation	*E. brunetti*
Caecum	Enlarged intestine filled with blood	*E. tenella*
Rectum	Inflammation, petechiae	*E. brunetti*

3 ▸ KOKCISAN IS A BROAD-SPECTRUM ANTICOCCIDIAL FOR POULTRY THAT FULLY CONFORMS TO THE REQUIREMENTS FOR AN ADVANCED HIGH-QUALITY FEED ADDITIVE

In broiler chickens, Kokcisan has been shown to be effective against the following strains of *Eimeria*: *E. acervulina*, *E. maxima*, *E. tenella*, *E. brunetti*, *E. necatrix*, and *E. mitis*. Kokcisan acts by preventing sporozoites to penetrate into the intestinal cells of the host. In those sporozoites that have already penetrated the intestinal cells, Kokcisan triggers numerous morphological changes. Kokcisan exerts a coccidiocidal effect at an early stage of coccidial life cycle, leading to the destruction of sporozoites, trophozoites and first generation schizonts. It does not have any influence on gametogony.
Kokcisan does not interfere with the development of immunity in infected broiler chickens.

The site of action of Kokcisan



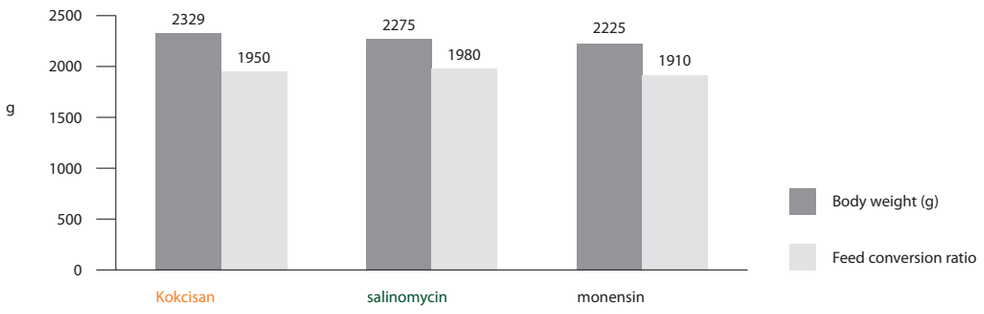
4 ▸ KOKCISAN ENSURES EFFECTIVE PREVENTION OF COCCIDIOSIS, INCREASING WEIGHT GAIN AND IMPROVING FEED CONVERSION IN BROILERS

COMPARATIVE STUDIES OF EFFICACY OF KOKCISAN AND OTHER COCCIDIOSTATS IN THE EU MEMBER STATES BETWEEN 1996 AND 2000

Trial in United Kingdom

The trial compared the results obtained in 3360 day-old Cobb broiler chickens, which were divided into three groups and fed either monensin (100 mg/kg), salinomycin (60 mg/kg), or Kokcisan (60 mg/kg).
The evaluation criteria included the clinical picture, mortality rate, body weight, feed conversion ratio (FCR) and efficacy of anticoccidial prophylaxis.
The birds were vaccinated with live Gumboro plus Newcastle disease vaccine at 21 days of age and a further live Gumboro vaccine at 34 days of age.

Results of the efficacy trials with Kokcisan on growth performance and FCR in broiler chickens in UK (0–42 days of age)

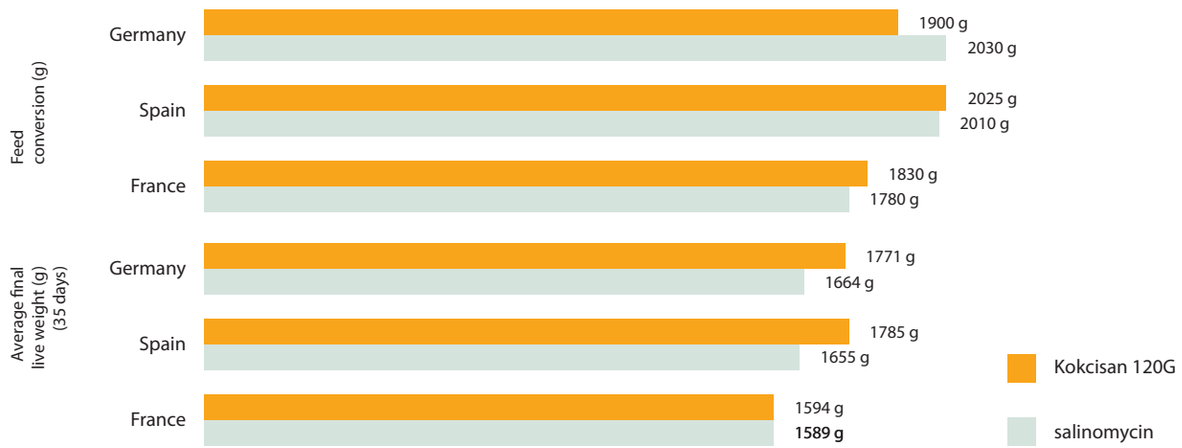


The statistical analysis indicated that the broilers given monensin had a 3% lower growth rate (P<0.01) than the birds given salinomycin or Kokcisan. There were no (P>0.05) treatment differences in the feed conversion ratio. There was no evidence of clinical coccidiosis in any treatment.

Trials in Germany, Spain and France

Trials carried out and analysed for the applicant in France, Spain and Germany, were conducted in conformity with the GCPV requirements.
The trials included a total of about 138,000 broiler chickens. They were carried out in different broiler integrations on different broiler farms, using Kokcisan 120G (60 ppm) and salinomycin (60 ppm) in the final feed for all the flocks.
The evaluation criteria included the clinical picture, mortality rate, body weight, feed conversion ratio (FCR), litter quality, lesion index, oocyst counting and statistical analysis.
Immunoprophylaxis: chickens were vaccinated against infectious bronchitis and against Gumboro disease.

Comparative studies showed no statistically significant differences (P>0.05) between the achieved production yield, the health condition and slaughter properties



In the study carried out in Spain, the body weight of chickens receiving Kokcisan was even higher, however, the results indicated that the differences were not significant (P>0.1).
Based on the available clinical data, it can be concluded that Kokcisan is a highly effective coccidiostat for broiler chickens at the recommended dose of 60 mg per kg of complete feedingstuff. Studies carried out under field conditions in different Western and Central European countries have shown that the product is effective against all economically important coccidial species in broiler chickens.

CLINICAL EFFICACY OF THE ANTICOCCIDIAL KOKCISAN COMPARED TO SALINOMYCIN, BY FECAL OOCYST COUNT AND AVERAGE LESION SCORE INDEX

Trial in France

	Oocyst counts (oocysts/g)	Average lesion score index
Salinomycin		
Day 21 (mostly *E. acervulina*)	30,600	0.70
Day 28 (mostly *E. acervulina*)	948,000	1.85
Kokcisan		
Day 21 (*E. acervulina* 65%, *E. maxima* 35%)	19,800	0.75/0.45
Day 28 (*E. acervulina* 82%, *E. maxima* 18%)	402,000	1.45/0.95

Trial in Spain

	Oocyst counts (oocysts/g)	Intestinal lesion scores
Salinomycin		
Day 28	–	20–0*
Day 35 (*E. tenella/E. maxima*)	200/400	16–4/1
Day 42 (*E. tenella/E. maxima*)	2800/400	–
Kokcisan		
Day 28	–	17–3/1
Day 35 (*E. acervulina/E. tenella*)	6200/600	16–4/1
Day 42 (*E. acervulina/E. tenella*)	400/600	–

Notes:
* = Number of chickens – score,
0 = no lesions, 1 = slight lesion, few hemorrhagic petechiae

Trial in Germany

	Oocyst counts (oocysts/g)	Average lesion score index
Salinomycin		
Day 21 (*E. acervulina*)	0	–
Day 28 (*E. acervulina*)	1950	0.8
Day 35 (*E. acervulina*)	3100	1.0
Kokcisan		
Day 21 (*E. acervulina*)	2350	–
Day 28 (*E. acervulina*)	1750	0
Day 35 (*E. acervulina*)	0	0

In different trials it was statistically confirmed that both anticoccidials had the same effect on the fecal oocyst count and on the average gut lesion score index. In the trial performed in Germany, Kokcisan appeared to be superior to the comparative drug, as shown by statistical analysis of lesion and parasite scores ($P<0.05$).
There are some differences relating to time (weeks of life); however, the results clearly show a good effect, relatively low oocyst counts, and a low average lesion score index, which ensure good final production results.

5 ▸ THE NEED FOR PROGRAMMED USE OF ANTICOCCIDIALS IN THE CONTROL OF COCCIDIOSIS

Programmed use of anticoccidial agents has become indispensable to broiler production, as subclinical coccidiosis causes increasingly higher indirect losses, which are manifested as poorer feed conversion and lower final body weight of chickens. An optimal protection against coccidiosis in the most delicate period of breeding (third and fourth week) is provided by a correct choice of anticoccidial agents. It is important to use drugs pertaining to different chemical groups, with a different mode of action on coccidia.
Anticoccidials are used in various ways. Some producers use programmes where an anticoccidial is used in the starter and grower feeds, and the finisher is unmedicated. In other instances, one anticoccidial is used in the starter and another in the grower feed. This method is called a shuttle programme. Shuttle programmes may involve two different ionophores, an ionophore and synthetic drug, or, rarely, two different chemicals. Some producers use more than two products in this type of programme, but this is not a widespread practice. If coccidiosis exposure is not high, such as in certain seasons of the year, producers may choose to reduce the length of time an anticoccidial is used. In some instances, the anticoccidial is withdrawn for two weeks. The decision to use such practice must be based on indi-vidual experience, along with the expected level of exposure for a particular locality and season (McDougald, 1998).

IONOPHORE ROTATIONS

The number of successive flocks for which a particular ionophore drug should be used in rotation programmes before changing to another compound is not known, but at least three are desirable. It is important that when a drug is changed, a compound with a different mode of action is chosen. The ionophores, and in particular salinomycin, monensin and narasin, have the same mode of action and should not be used as alternatives in rotation programmes. The ideal rotation programme should involve a switch from an inonophore to a compound with an entirely different mode of action, for example diclazuril, halofuginone, nicarbazin, or robenidine.

HOW TO USE KOKCISAN?

Kokcisan can be used in full and shuttle programme. When utilising the full programme (administration of an anticoccidial over several consecutive cramming periods), Kokcisan can be used for six months at maximum.

It is recommended that the shuttle programme should not last longer than six months. When utilising the rotation programme, Kokcisan may just as other ionophores be used for a maximum of 3 to 4 subsequent flocks. The recommended time of use of Kokcisan is based on experience obtained in different European countries. However, the exact programme on a specific farm, poultry integration or even country area, should be based on local epizootiological situation, previous use of salinomycin or other drugs, climate conditions, type of rearing technology (such as density, litter removal, equipment), and other factors.

6 ▸ THE SAFETY PROFILE OF KOKCISAN

At recommended concentrations (60 ppm), Kokcisan has not been associated with any side effects. Practical experience of professionals working on poultry farms shows that negative effects on weight gain and feed conversion occur at doses exceeding 90 ppm, although this value in itself is not associated with any clinically manifested toxic effects. In our studies, such negative effects were registered only at values of 150 ppm. Based on practical experience and our own results, a definite conclusion can be made that the recommended concentration of 60-70 ppm is absolutely safe.
Tolerance of Kokcisan was evaluated in broiler chickens after feeding birds (from 1 to 56 days of age) with a diet containing the product at doses that corresponded to salinomycin concentration of 60, 120 and 150 ppm. The results of the study showed that Kokcisan admixed in the feed had no effect on the health of broiler chickens when given at the recommended incorporation rate (60 ppm), or at twice the recommended incorporation rate (120 ppm) for 56 consecutive days. But when the birds were given feed containing 2.5 times the recommended incorporation rate, i.e. 150 ppm, a marked reduction in feed consumption and growth rate was registered.

Potential toxic effects of Kokcisan may result from the following:

- application of doses considerably exceeding the recommended concentration
- extremely nonuniform distribution of Kokcisan in the final feed (due to technological errors when admixed to feedstuff)

Kokcisan tolerability has been shown to vary between different animal species. Especially dangerous side effects may occur in horses and other equines, and turkeys.

7 ▸ ENVIRONMENTAL IMPACT

The additive is metabolised into products with minimal biological activity. The major residue consists of the molecule and its metabolites. Salinomycin excreted in the faeces is rapidly degraded in soil. The product is harmful to daphnia and toxic to fish and algae. Salinomycin presents no risk of bioaccumulation and is not absorbed by plants.

8 ▸ COMPATIBILITY

Kokcisan is compatible with all approved feed additives and veterinary pharmaceuticals except tiamulin.

9 ▸ INCOMPATIBILITIES

Salinomycin is reported as interactive in poultry with chloramphenicol, erythromycin, sulphachlorpyrazine, sulphadimetoxine, sulphadimidine, sulphaquinoxaline and tiamulin.

10 ▸ DETERMINATION OF KOKCISAN IN THE FINAL FEED

High performance liquid chromatography (HPLC) is an accurate and specific method for determination of salinomycin in vitamin-mineral premixes and in complete feed mixtures. The HPLC method is equally suitable for both the quantitative and qualitative determinations in vitamin-mineral premixes and in the final feed. The amount of Kokcisan could be detected up to 1 ppm.

11 ▸ TECHNICAL DATA

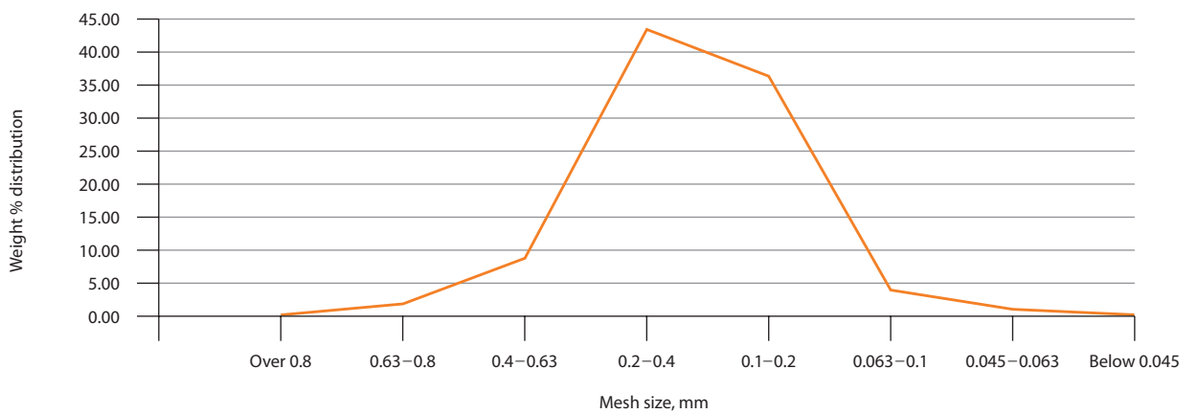
DUST FORMATION

Dust formation is an important feature of feed additives, since in mixing plants the emission of dust particles may be the cause of cross-contamination and allergic reactions in the staff. In addition, it has a negative impact on the overall anticontamination measures.
The dusting potential of Kokcisan as determined by the Stauber-Heubach method amounts up to 0.8 g/m^3. Such dust behaviour is recognised as satisfactory and is in the range of commonly used feed additives.

PARTICLE SIZE DISTRIBUTION

Particle size distribution determined by sieve analysis, is declared to be between 0.1 and 0.4 mm in more than 80% of the particles, and shows good correlations with particle size of premix and final poultry feed (Institute of Feed Technology, IFF, Braunschweig, Germany), facilitating satisfactory mixing ability.

Particle size distributions



DISTRIBUTION IN PREMIXES AND FINAL BROILER FEEDS

Kokcisan is well distributed in premixture and final feed; salinomycin sodium coefficient of variation ranges between 3 and 7% at the recommended concentration of 60 ppm. No segregation tendency during the manufacturing of premixture or feedingstuff was observed. Particle size distribution of common broiler floury feed ensures a homogeneous distribution in feedingstuff as well as homogeneity and stability under manufacturing and handling conditions. After adjusting the obtained result by analytical and sampling methods of variance coefficients, it was demonstrated that the distribution of Kokcisan fully complies with the accepted requirements according to which the maximum deviation is ±20%. This standard is accepted by European countries, the USA, and worldwide.

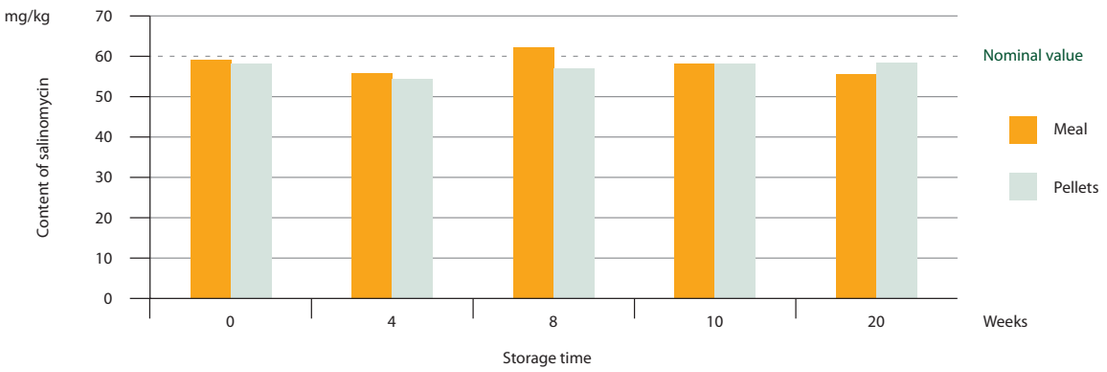
STABILITY

Kokcisan stored in original packing is stable for 2 years.

STABILITY IN FINAL FEED

By testing the storage properties of the broiler premix and final feed in powder and pellet form, it was confirmed, by a 20-week analysis and statistical evaluation, that Kokcisan is highly stable. At the 60 ppm content, which is the recommended quantity in the final feed, salinomycin maintains its stability for at least 20 weeks (IFF, Germany).

Long-term stability



12 ▸ INSTRUCTIONS FOR USE

ADMIXING INTO FEED AND DOSAGE

Kokcisan is recommended for in-feed use in starter or grower/finisher diets for poultry and may be used prophylactically throughout the fattening period of broiler chickens. When used in broiler chickens, the final mix should contain 60-70 mg of salinomycin sodium per kg. This can be achieved by incorporation of 500-583 g Kokcisan per tonne of finished feed to provide a concentration of 50 to 70 ppm in the final feed. To ensure homogeneity, the preparation of intermediate premixture containing 0.6–1.2% salinomycin sodium is recommended. The finished feed assay should be within the common accepted limits of ±20% of the target value. In EU, the dose ranges between 50 and 70 ppm, in accordance with the importance or incidence of coccidiosis in poultry integrations.

WITHDRAWAL PERIOD

Birds must not be slaughtered for human consumption during treatment and for at least three days after the last treatment.

SAFETY RECOMMENDATIONS REGARDING THE USE

Dangerous for equines and turkeys.

This feedingstuff contains an ionophore: simultaneous use with certain medicinal substances (e.g. tiamulin) can be contraindicated.

Chickens for fattening should not be treated with products containing tiamulin while receiving, or for at least seven days before or after receiving feed containing Kokcisan 120G. Severe growth depression or death may occur.

STORAGE

Kokcisan should be stored in original packaging in a dry and dark place at a temperature below 30°C. It should be protected from moisture and light.

PRECAUTIONS IN HANDLING OF KOKCISAN

Strict protection measures are recommended in the production plant: ventilation of premises, wearing of overalls, gloves and glasses and a face mask with a respiratory filter for the protection of operators to avoid direct contact with the product or inhalation.

SUPPLY

25-kg net in polythene-lined multi-walled paper bags.

Salinomycin is an antibiotic and coccidiostat from the ionophore family, which is not used in human medicine or veterinary therapy and the chemical formula of which bears no similarity to those of other antibiotics molecules currently in use.

KOKCISAN® 120 G

12% granulate

salinomycin sodium

- is a broad-spectrum anticoccidial for poultry that fully conforms to the requirements for an advanced high-quality feed additive.
- is your best partner in anticoccidial programmes for intensive broiler production, ensuring effective prevention of coccidiosis, increasing weight gain and improving feed conversion.
- is produced by fermentation from the culture of *Streptomyces albus* in accordance with the GMP, GCPV, HACCP, ISO 9000 and 14000 standards.
- is stable in premixes and in the final feed.
- does not affect feathering, skin pigmentation and meat quality after slaughter.

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4/2008, United Kingdom, 2008-6931, SEDNA/ARTUM.