SEK 12

Lyttyetti (Summary)

THE INGESTION OF ANTIOXIDANTS BY THE PIG AND THE CHICKEN.

THE EFFECT ON THE COMPOSITION OF CERTAIN TISSUES AND ON THE STABILITY OF STORED FATS.

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The use of antioxidants in animal feeding is, at present, evisaged for several purposes : on the one hand the use of some of these substances such as DPPD or BHT, has been suggested for the prevention of poultry encephalomalacia. On the other hand, antioxidants can be used to stabilize the fats in animal feedstuffs, whether the fats are the natural fats of the diet or those which are added in order to enhance the energy value of the ration. It is known, however, that the oxidation of fats in nutrients is liable to cause, in the ingesting animals, nutritional accidents resulting directly or indirectly from the presence of the oxidation products of the fats.

Certain antioxidants administered per os to animals are capable of accumulating in various tissues. For example, research workers at the University of Minnesota (BARNES & Coll., LUNDBERG & coll., HANSON & Coll.) have shown that the stored fats of the rat are stabilised when tocopherols are added to the diet, where as the addition of hydroquinone provokes, on the contrary, a reduction in the stability of these fats with regard to oxidation.

Numerous synthetic antioxidants are at present suggested for animal feeding. Amongst these gallates, NDGA, BHA, BHT, and DPPD are the most usual. The investigations which were the object of this note aimed at studying the effect of the ingestion of these antioxidants by the pig and the chicken, on the eventual accumulation of these products in the various tissues and organs, of the animals, on the one hand, and on certain compositional characteristics on the stored fats, on the other.

Experimental procedure

Antioxidants used - animals

The following antioxidants were studied : propyl gallate, dodecyl gallate, butylhydroxytoluene (BHT) or dibutylparacresol (DBPC) (two commercial forms of this product were studied, norhydroguaiaretic acid or NDGA, butylhydroxyanisole or BHA, diphenylparaphenylenediamine or DPPD. The dose incorporated in the feeds was 0.1 % for all products except DPPD which was administered at a level of 0.125 %. This amount is considerable and corresponds to 10 times that which is generally recommended for the stabilization of fats.

For pigs, the administration was continued for about 4 months (weigt 50-100 kg). Each set, including one control set, consisted of 5 animals. With chickens, the experiment lasted 8 weeks. Each set comprised 15-25 birds.

Organs and tissues studied

In the pig, the dorsal fat was sempled at slaughtering. The two layers which make up this tissue were separated and analysed individually. In

addition, the perinephrite fats (panne) were sampled. These fatty tissues were melted in an atmosphere of CO_2, filtered and centrifuged. The following determinations were carried out on these fats : level of antioxidants, peroxide index, a stability test and iodine index. The stability test was carried out in an illuminated oven at 60° according to a method recommended by DESNUELLE and by WOLFF. The semi-tendinous muscle, the liver and kidney were also sampled for analysis. In the chicken, stored fats were often lacking, even though the animals were kept for 16 weeks in order to obtain sufficient fattening. We have therefore been unable to study individual animals. In fact, in order to obtain sufficient fat, we joined up samples from two or three animals. For the muscular tissues, the pectorals and the claw muscles were sampled.

Method of determination.

a) Fats

The iodine indexes were determined by the method of WIJS and the peroxide indexes (LEA) by iodometry.

b) Antioxidants

The method of KAHAN was used for determining NDGA, BHA and BHT. We also used this method for the determination of gallates. In fact, the ration of the animals contained only one antioxidant and therefore the lack of specificity of the reaction with the reagent, ferric chloride-dipyrydile, was not a drawback. However, we realized the necessity of drawing a calibration curve for each series of determinations, using this reaction, whatever the antioxidant being determined.

For DPPD the nitro derivative was prepared and determined colorimetrically (BUNNEL). To determine this antioxidant in fats we had to carry out a preliminary saponification in the cold, having previously ascertained that this did not modify the validity of the determination.

The extraction of the antioxidants from tissues and organs poses certain problems. In particular, BHT, according to the method of ANGLIN, MAHON, and CHAPMAN, should be distilled with superheated steam. We found, however, that for quantitative distillation the temperature of the distillation should reach 180°.

In the case of the fatty tissues, these are dissolved in petroleum ether. The antioxidant (BHA, NDGA, Gallates) is reextracted in 72 % alcohol. For muscle, BHA and gallates are extracted in the Blendor by the method of HANLEY. On the other hand, the presence of gallates at extremely low concentrations can be detected by the addition of a few drops of concentrated ammonia (WENGER).

Results

A. Validity of the methods of determination.

Overdosing tests were carried out with the different antioxidants, in order to determine the lower limit of sensitivity as well as the accuracy of the determination. The overdosing was carried out on fatty tissue, muscle, liver and kidney. The concentrations used varied from 0,001 % to 0,1 %.

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a) Fat

For several antioxidants the degree of recovery reached values in the region of 95 %, even at concentrations as low as 0.001 %.

However, the recovery of propyl gallate and BHT is lower. In the latter case the determination procedure, which involves distillation with superheated steam, is probably responsible for the relatively low value. In no case were we able to confirm the results of ANGLIN and coll. When we carried out in detail the method proposed by these authors, without altering the temperature of distillation, we did not obtain recoveries greater than 50-60 %.

Be that as it may, a degree of recovery of the order of 85-90 % is amply sufficient to determine whether a stored fat does or does not coutain an antioxidant.

	External lard	Internal lard	Panne	Muscle	Liver	Kidney	Blood
Propyl gallate	nil	nil	nil	nil	nil	nil	
Dodecyl gattate	-	-	-	-	-	-	-
B.H.A.	-	-	-	-	-	-	-
N.D.G.A.	-	-	-	-	-	-	-
B.H.T.	-	-	-	-	-	-	-
D.B.P.C.	-	-	-	-	-	-	-
D.P.P.D. (1)	0 à 0,0025	0 à 0,0025	0,0012 à 0,0025	0	0	0 à 0,0006	

Table I

Antioxidant content of the tissues of the pig

The ration contained 0.1 % of the antioxidant studied, except for DPPD which was added at a level of 0.125 %

Duration of the administration of the diet : about 4 months

(1) See the detailed table for this antioxidant

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Table II

The DPPD content of various tissues of the pig
(individual values) gr per 100 gr

Pig	External lard	Internal lard	Panne	Muscle	Liver	Kidney	Blood
A	0,0022	0,0024	0,0024	0	0	0,0006	0
B	0	0,0014	0,0016	0	0	0,0006	-
C	0,0025	0,0024	0,0023	0	0	0	-
D		0,00094	0,0012	0	0	0,0005	-
T	0	0	0,0023	0	0	0,0005	-
U	0,0022	0,0015	0,0024	0	0	0,0006	-

Table III

The average antioxidant content of the tissues of the chicken * (in g. %)

	Fat	Muscle	Liver
Propyl gallate	0	0	0
Dodecyl gallate	-	-	-
B.H.A.	-	-	-
N.D.G.A.	-	-	-
B.H.T.	0,006	?	?
D.P. P.D.	0,0007	-	-

*Level of antioxidants in the diet : 0. 1 % except for DPPD (0.125 %)
Age of animals : about 16 weeks
Duration of treatment : 8 weeks

Table IV

The DPPD content of various tissues of the chicken
(in g. %)

Groupe	Fat	Muscle	Liver
9	0,001	0	0
10	0,0005	-	-
11	0,0007	-	-
12	0,0005	-	-

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b) Muscle and organs

Recoveries are also often of the order of 95 %, as regards the muscle. For the kidney and liver, certain difficulties present themselves with regard to DPPD and propyl gallate. In the end we had to abandon the determination of BHT in the tissues by the method of ANGLIN and Coll. The superheated steam carries over reducing volatile substances which render impossible the specific determination of BHT.

B - The movement of antioxidants in the tissues.

The methods studied were applied to the different tissues and organs sampled under the conditions mentioned above.

I - Pig

The results in Tab. 1 show that the antioxidants studied do not accumulate in the adipose tissue, the muscle, liver, kidney or blood. It should be noted that we investigated gallic acid also, since an in vitro trial showed us that gallates are rapidly hydrolysed by the contents of the intestine of the pig. For example, after incubation in this environment, it is possible to find, BHA quantitatively where as gallates disappear completely. The qualitative tests for gallic acid ($FeCl_3$), when applied to fatty tissues and organs were always negative.

One antioxidant, however, is an exception : diphenylparaphenylenediamine (DPPD).

The level of DPPD found in the lard and in the panne is always low in exact figures : 0,0025 % at the most, that is 25 milligrammes per kg of fat. It should be noted that this low concentration never the less suffices, in some cases, to impart a faint rose colouration to the fat. We shall see, however, that this concentration is also sufficient to retard oxidation considerably.

Individual variations occur in the quantity of antioxidants contained in the fat. (Tab. 2). Amongst the other tissues and organs, the Kidney is capable of retaining DPPD, the concentration found is of the order of 0.0005 % whereas neither the muscle, the liver, nor the blood contain determinable quantities of antioxidants.

II - Chicken

a) Fats

The study of the tissues of the chicken, summarized in tab. 3 and 4, shows that two antioxidants are capable of accumulating in the fat of these birds, DPPD and BHT. However, the level of DPPD found in the chicken (0.0007 %) is lower than that found in the pig. The concentrations are 2 to 3 times weaker in the former than in the latter.

In confirmation of this result we noticed that the fats of treated birds are not stabilized, whereas there is a markedly increased period of induction in the case of the pig.

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On the other hand BHT (or DBPC), which we could not detect in the fat of the pig, exists in chicken fat at a concentration which is easily determined (0.006 %). We ascertained, in the control animals, that the fat of the chicken contained no interfering substances. Finally, we obtained confirmation of this determination by a study of the stability of the stored fats. The induction period of the fats of the animals which had received BHT was markedly increased. Test of addition of BHT, in known quantities, to the fats of control chickens showed that, for similar doses, the increase in stability was of the same order of magnitude as that which we found by direct determination.

C - Influence of the ingestion of antioxidants on the stability of stored fats, with regard to oxidation.

The stability of stored fats with regard to oxidation, is the result of two principal opposing factors. On the one hand the level of unsaturated acids, and on the other, the level of antioxidants.

As far as concerns the former of these two factors, we have determined the iodine index for each sample, which as a first approximation gives a measure of the quantity of unsaturated fatty acids present in the fat.

The results of the determinations of the antioxidants have already been given above. In addition we have used an indirect method which consists in studying the stability of the fats with regard to an artificially accelarated oxidation. One thus studies the kinetics of the development of the peroxide level of the fats. The samples taken from animals receiving antioxidants were compared on the one hand with the fats of the control animals (without antioxidant) and, on the other hand, with samples of fat from control pigs, to which known quantities of each antioxidant were added. By a comparison of the duration of the latent periods, it is thus possible to determine the magnitude of the antioxidant content of the fatty tissue.

The average iodine indexes of the pig fats studied are summarized in the following table :

	External lard	Internal lard	Panne
Control	62.3	55.5	48.4
BHT (1)	62.5	56.9	47.5
BHT (2)	64.2	58.7	49.4
BHA	62.7	56.6	49.6
Propyl gallate	61.4	55.5	49.3
Dodecyl gallate	65.2	61.1	51.8
NDGA	62.8	57.0	49.2
DPPD	64.7	57.3	47.7

The indexes are not markedly altered in any set. The addition of antioxidants to the rations does not therefore run any risk of altering the melting point of the fats. It should, nevertheless, be noted that a significant difference exists between the average iodine index of the control set and the

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set receiving DPPD. There has therefore been a "sparing" of the unsaturated fatty acids in the animals ingesting this antioxidant. It should be noted that this observation has already been made by BRATZLER, who studied the composition of pig fat. In the animals receiving tocopherols there was an accumulation of oleic acid at the expense of the saturated fatty acids. It should be remembered, however, that GARTON found no such effect.

1) Pig

Fig. 1 shows the development of the peroxide index of fats kept at 60°. We have calculated the average duraction (in hours) required to reach peroxide indexes equal to 5, 10, and 20. This figure refers to internal fat. Fig. 2 and 2a indicate the development of the control fats to which known quantities of antioxidants were added : 0.001 % to 0.0001 %.

The first graph shows very clearly that the DPPD ingested by the animals stabilizes considerably the stored fats with regard to oxidation. Fig. 2 shows that the addition of 0.001 % DPPD enables a peroxide index of 10 to be reached in c. 200 hr. The value of 20 is reached in 300 hr. This is of the same order of magnitude as values shown in fig. 1. It can be concluded that the concentration of DPPD is of the order of 0.001 %. It should be remembered that direct determination gave a value of between 0.0012 and 0.0025 %. The study of the kinetics of the peroxide level confirms, therefore, the result obtained by direct determination.

The internal lard of the set which received BHT appeared to be slightly stabilized in comparison with other sets. A study of fig. 2 also shows that BHT added to fats at a concentration of 0.001 % leads to the appearance of a peroxide index of 20 after 150 hr. Nevertheless, a comparison of fig. 1 and 2a enables one to conclude that the level of antioxidant eventually stocked in the fat would be less than 0.001 % and probably in the region of 0.0001 % (an index of 20 after 80 hr.). Consequently, one can presume a slight movement of BHT but in quantities too low to be shown up by chemical determination.

For the other antioxidants it is impossible to show the occurence of a significant stabilization of the fats. But, if such a stabilization exists it could be indirect, in fact, the synthetic antioxidants added to the diet could provoke a "sparing" of natural antioxidants such as the tocopherols. Nevertheless, in the case of synthetic antioxidants, direct determination in the tissue avoids such an ambiguity.

2) Chicken

The iodine index of chicken fats is markedly higher (67 to 72) than that of the pig fats. On the other hand, the level of DPPD is three to four times lower than that in the pig. These two differences alter the effectiveness of the antioxidant. In fact, the fats of the chickens treated with DPPD are practically not stabilized at all. On the contrary, BHT, which we have determined at levels of 0.006 % brings about considerable stabilization of the chicken fats (fig. 3). The two methods, direct and indirect, thus give similar information.

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Discussion

The results obtained in the course of these studies have enable one to show clearly the movement of certain antioxidants in animals. The movement is connected mainly with the fatty tissue, in which, in the pig, DPPD alone is in practice able to be stocked. In the chicken, DPPD is equally capable of crossing the intestinal wall and becoming stocked in the fats. Similarly for BHT, but he concentration of this antioxidant reaches values very much higher than those for DPPD. The levels found are, in exact figures, nevertheless very low, of the order of 0.005 % at the maximum.

It should also be stressed that the level of 0.1 % or 0.125 % of antioxidant incorporated in the feeds is 10 times greater than that recommended for the stabilization of the fats. Nevertheless, the stabilization of certain nutrients (animal meals in particular) can require high concentrations. Likewise the levels of incorporation of the antioxidants in the nutrients for poultry, in order to prevent encephalomalacia can be relatively high. However, the concentrations which we used constitute a maximum which would not be attained in practice. Under these conditions, one can presume that the levels found in the tissues would be considerable lower than those which we found.

But the stabilization of stored fats by means of nutritional antioxidants poses a problem with a double aspect. From the point of view of the technology of animal products this stabilization is undoubtedly a favourable factor. But, from the point of view of the hygienist, it is necessary to ascertain that the antioxidant present in the tissues is of no harm to the consumer of the pig or chicken meat. The toxicity of DPPD is known (AMES), whereas BHT is but very slightly toxic in the body (TEICHMAN).

Summary

The effect of ingesting various antioxidants has been studied in the pig and the chicken. The animals received 0.125 % DPPD or 0.1 % BHA, propyl gallate, dodecyl gallate or NDGA in their ration. The content of antioxidant in the muscle, fats, liver and kidney were studied. In the pig, only DPPD was determinable in the stored fats (maximum : 0.0025 %) and in the kidney (maximum : 0.0006 %) of the treated animals.

In the chicken DPPD is found in fat at a lower concentration (0.0007 %) than in the fat of the pig. On the other hand BHT is stocked in the fat of the chicken at a level of 0.006 %.

A study of the stability of the fats of control and treated animals confirmed the results of direct determinations of antioxidants. In fact, the fats of pigs receiving DPPD and those of chickens receiving BHT had a much longer induction period.

The technological and hygienic consequences of these results are stressed.

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Figure 1

The development of the peroxide index
of the samples of internal lard
(the animals having ingested the different antioxidants)

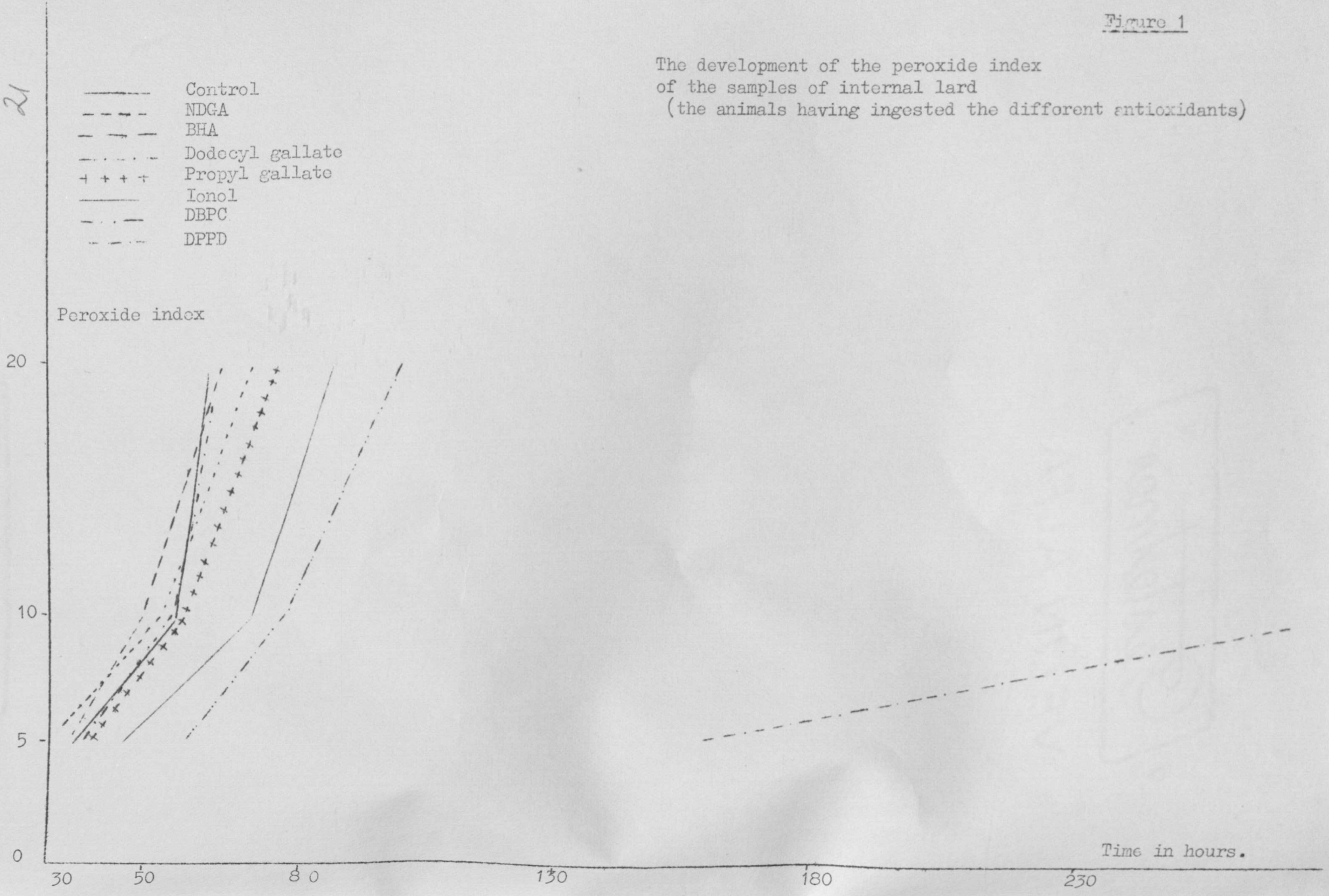


Figure 2

Internal fat of the pig to which 0.001 % of each antioxidant had been added

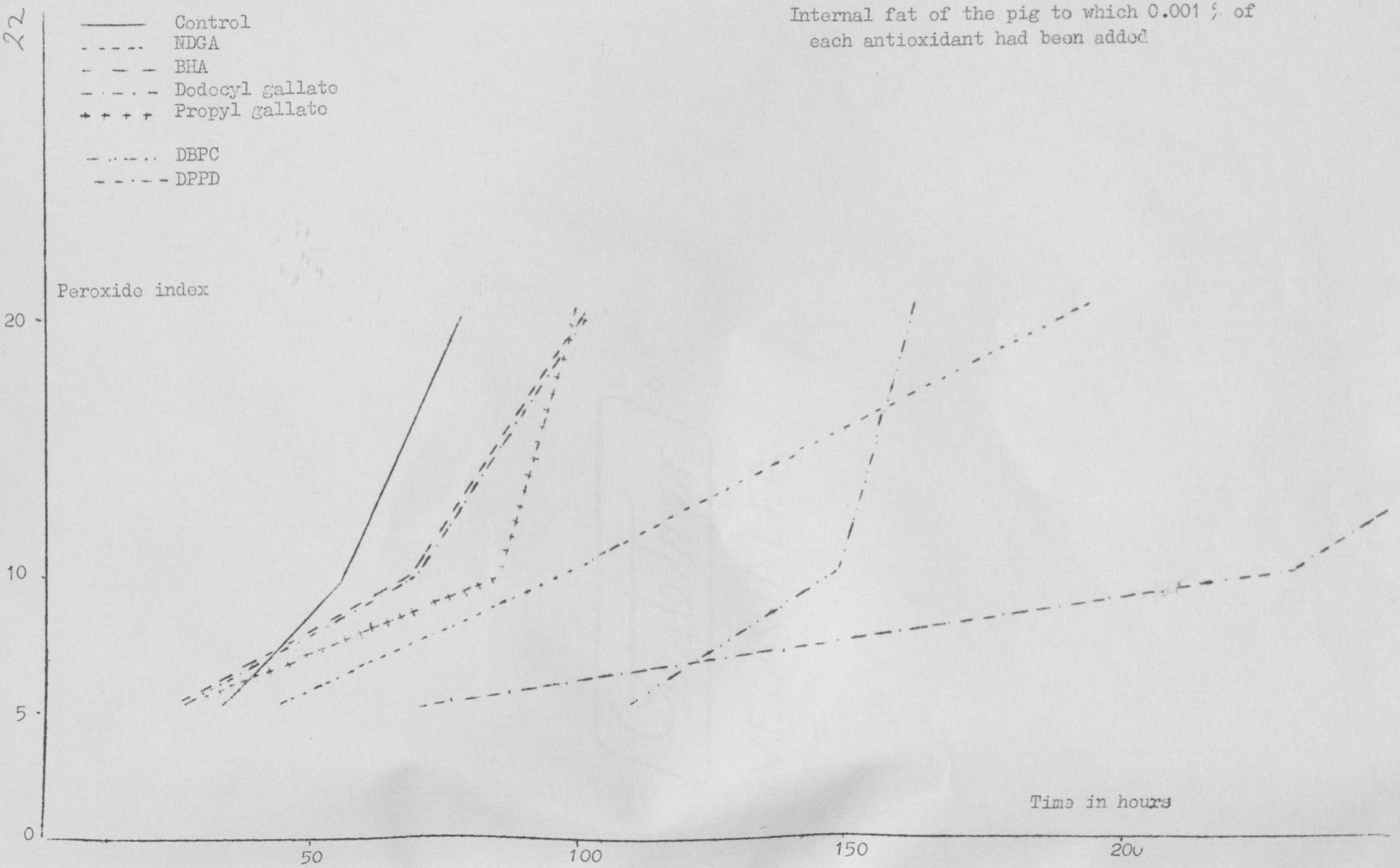
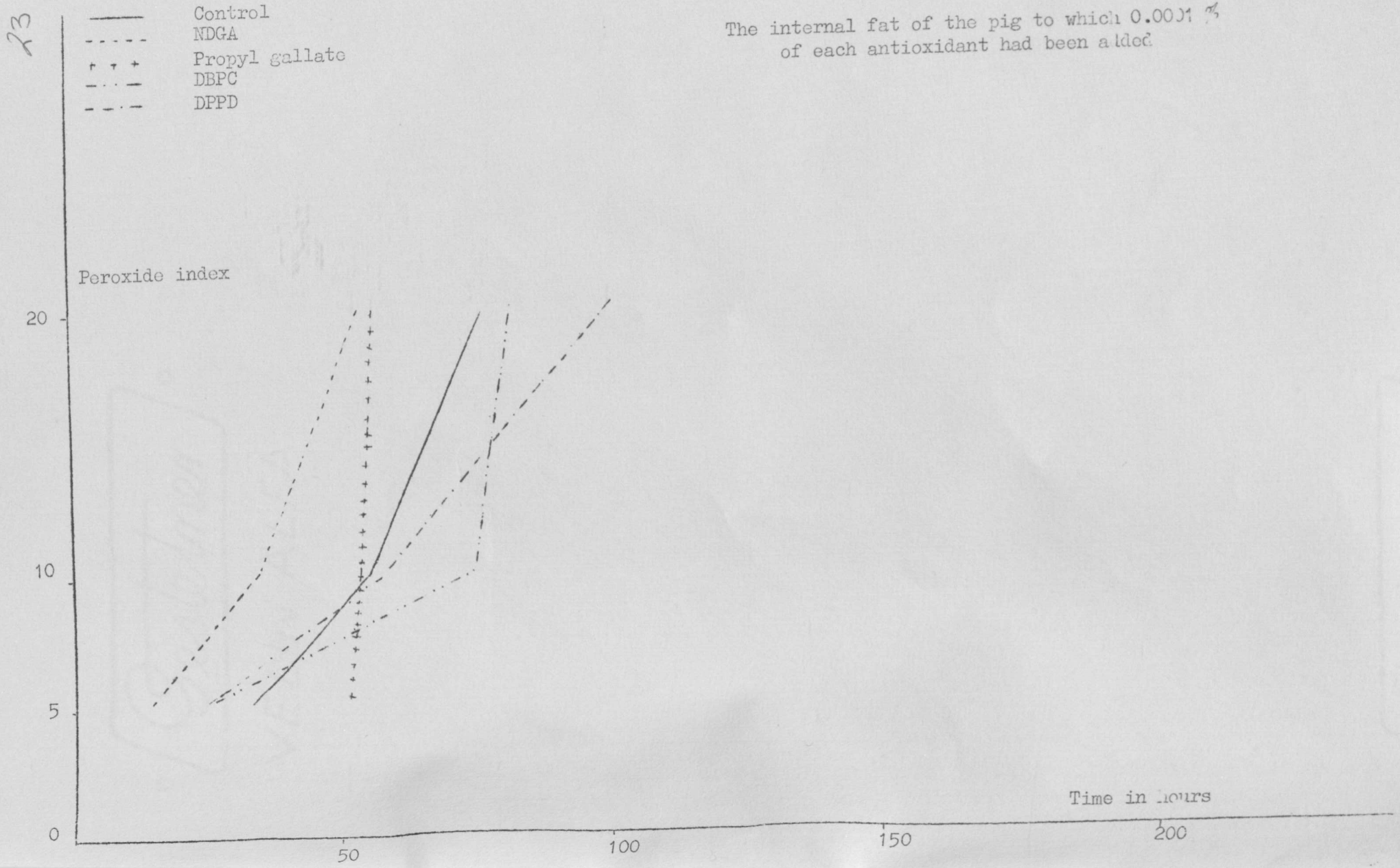
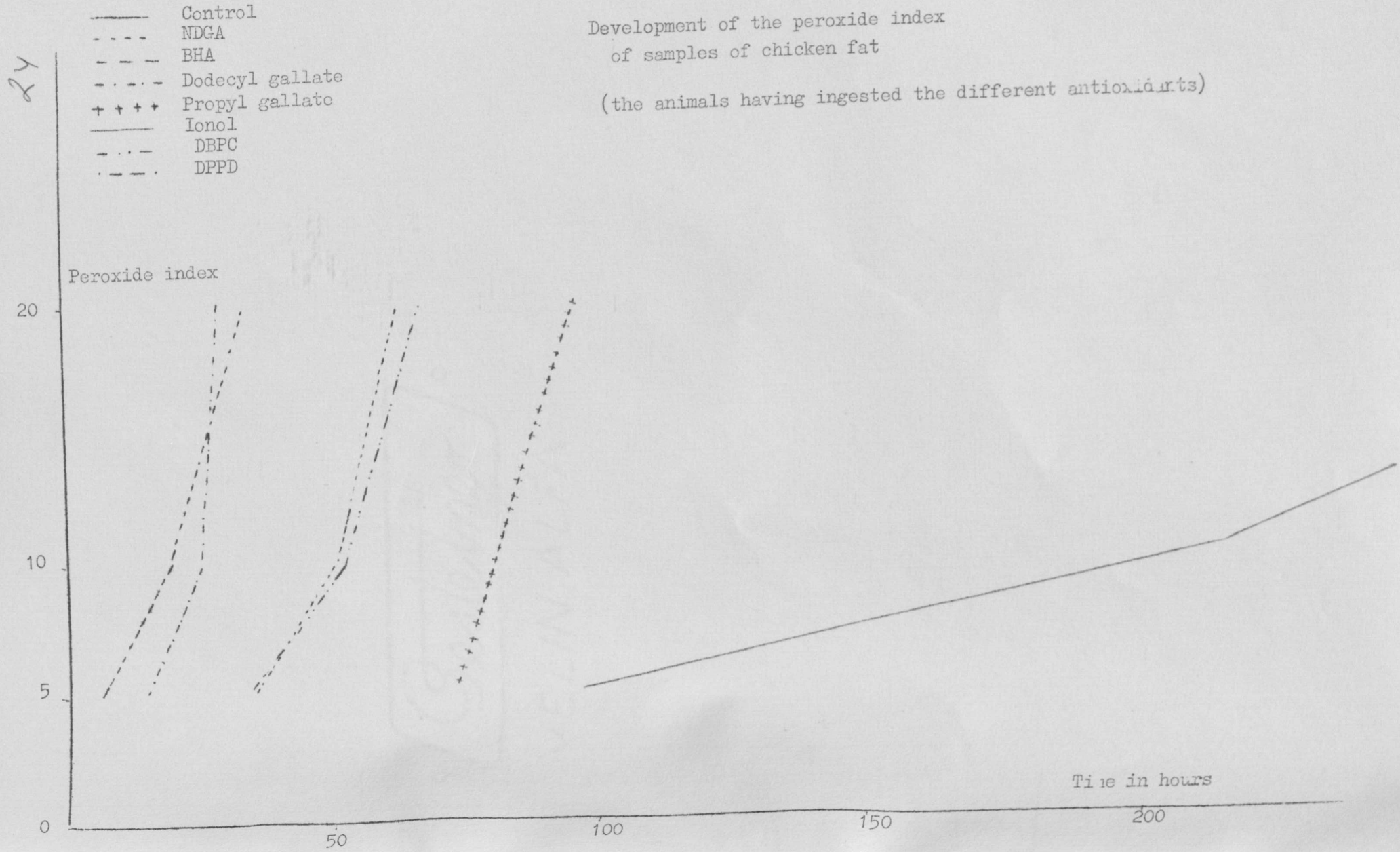


Figure 2a



The internal fat of the pig to which 0.0001 % of each antioxidant had been added

Figure 3



Development of the peroxide index
of samples of chicken fat

(the animals having ingested the different antioxidants)

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