

A Rapid Phase Test for distinguishing between Carotinoid and Bile Staining of Fat in Carcasses.

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In the routine inspection of carcasses a yellow discolouration of the fats is not infrequently observed, but the significance of this discolouration from a meat inspection point of view depends upon whether it is caused by the impregnation of the tissue with bile (icterus) or is due merely to the presence of harmless, or even beneficial pigments of vegetable origin (pseudo-icterus). Bile is not normally present in significant quantity in the blood or tissues of the healthy adult pig, sheep or bovine and, therefore, the presence of icterus in any of these species can be said to imply a pathological state.

Green plants contain, associated with their chlorophyll, yellow pigments belonging to the class known as carotinoids and these latter being easily absorbed and fat soluble tend to accumulate in the adipose tissues when an animal is liberally supplied with green food or materials such as yellow maize which also contain appreciable quantities of the pigments.

Although this is the case peculiarities are shown by certain species, the fat of the pig, for example, being usually almost colourless while bovine fat, absolutely free from carotin, is rarely encountered. Furthermore one sometimes finds that in bovines this yellowish discolouration is not confined to fatty tissues but can occasionally be recognised clinically as a yellowish discolouration of the visible mucous membranes.

Carotin discolouration of fat is in no way detrimental, the only support which could be advanced for the popular prejudice against such highly coloured carcasses being the fact that carotin pigmentation of the fat appears to increase in intensity with age.

In abattoir practice, however, it becomes necessary to distinguish between bile and carotin staining so that icteric carcasses may be rejected and excluded from sale. For this purpose one or other of the following methods is customarily employed.

1. Visual examination of colour quality. It is said that carotin staining imparts a colour to the fat of a clear yellow as against the more dirty yellow of bile discolouration.
2. The hanging test. A suspected carcass is allowed to hang in the chilled room for 24 hours. Carotin pigmentation is said to decrease and disappear entirely under these conditions, whilst bile pigmentation remains unchanged.
3. A chemical test is performed such as that put forward by Martin or van Manen.

Subjecting to scrutiny each of these methods in turn, it may be said that the first whilst valuable in the hands of an experienced inspector is nevertheless unsatisfactory because it is empirical and lacks precision. Mistakes might conceivably be made in which both diseased carcasses are passed (the import inspector who has not the viscera available is placed at the greatest disadvantage) and, perfectly healthy carcasses condemned because they show a harmless yellow discolouration, mistaken for icterus.

The hanging test is equally unreliable since on the one hand carotin pigmentation may persist for weeks or even months and on the other bile staining has been known to disappear completely overnight (van Manen 1933).

No chemical test so far proposed allows of a rapid result being obtained. Martin (1931) recommends the preparation of an alcoholic extract of the fat to be examined by shaking this with 96 per cent. alcohol for $\frac{1}{2}$ to 2 hours. After filtration a few drops of sulphuric acid are added and the mixture heated when the presence of bile is indicated by the appearance of a greenish colour (due to biliyerdin). Van Manen's method is really a modification of Martin's test, practically the same procedure being followed to obtain an alcoholic extract but this is then treated with the Ehrlich diazo reagents when the familiar reddish colour of azo-bilirubin appears.

Both these methods afford information concerning the one pigment only, are time consuming and elaborate and consequently would appear to be only infrequently used.

In addition it may be pointed out that the carotinoids themselves afford brilliant blue colours with strong mineral acids. Thus uncertainty might still be entertained that under the conditions of Martin's test a bluish colour could be due to carotin and not to bilirubin.

It was, therefore, felt that a simple and rapid test to distinguish between bile and carotin staining and which unlike the other tests affords at the same time information concerning the absence or presence of one or the other or of both pigments, would be of considerable value in ordinary abattoir practice. It is claimed that the test to be described below conforms to these requirements.

This test is an extension of that evolved recently (Rimington, 1937) for the examination of sera and is named the phase test since two liquid layers are produced, the upper (ether) one containing any carotinoids present and the lower (sodium hydroxide) retaining the bile pigments. It is performed as follows:—

The fat to be examined should be as free as possible from blood; renal fat is generally very suitable. About 2 gm. is placed in a strong glass test tube with approximately 5 c.c. of a 5 per cent. aqueous solution of sodium hydroxide and the mixture is heated over a flame so that boiling is maintained for about 1 minute. Agitation of the tube aids the disintegration of the fat which takes place during this procedure. The tube is now cooled under the tap until only comfortably warm to the hand (40-50°C). One half to an equal volume of ether is now added and the contents of the tube mixed by careful agitation and then allowed to separate into the two layers. Should persistent emulsion prevent the rapid separation of the phases, this may be overcome by warming gently or by adding a few drops of alcohol.

Since bilirubin forms a water soluble sodium salt it remains in the lower phase which is thereby coloured greenish yellow, the intensity depending upon the quantity of bile which is present. The upper phase will contain any carotinoid pigments, these imparting to the ether a yellow colour. Naturally various combinations are possible, bile alone, carotin alone or mixtures of the two or absence of both and the test will indicate these by a colourless upper and greenish-yellow lower phase, a colourless lower and yellow upper phase, or lastly yellow above and greenish-yellow below or no colour at all. Trial showed that when added to a colourless pork fat, as little as 0.05 c.c. of bile could easily be detected when performing the test.

The chief advantages claimed for the phase test are that it is rapid, requires no special apparatus or chemicals and affords indication of the presence or absence of both carotinoid and bile pigments. By its use decisions based upon reliable criteria could be made with a great saving of time.

REFERENCES.

- MARTIN, I. (1931). Detection of Bile pigments in tissues of icteric slaughter animals by a simple chemical reaction. *Zeit f. Milch.u.Fleisch-Hygiene*, Vol. 41, pp. 497-9.
- RIMINGTON, C. (1937). The occurrence of carotinoids in animal sera: The danger of mistaking carotinaemia for bilirubinaemia. *J.S.A. Vet. Med. Assoc.*, Vol. 8, No. 2, pp. 7-9.
- VAN MANEN, A. (1933). The demonstration of bile pigments in tissues of slaughter animals. *Tijdschrift voor Diergeneeskunde*, Vol. 60, pp. 449-457.