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RESEARCH NOTE

PRODUCTION OF A CHICKEN MEAT INFUSION BROTH SUITABLE FOR THE MASS PRODUCTION OF A *HAEMOPHILUS GALLINARUM* BACTERIN

S. B. BUYS, Veterinary Research Institute, Onderstepoort, South Africa, 0110

ABSTRACT

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A chicken meat infusion broth was prepared by various formulations and tested in 200 ℓ volumes for their suitability to support the growth of *Haemophilus gallinarum*. The best yield (0,1% packed cells, or 10^9 colony-forming units/mℓ) was obtained with a medium prepared from whole flayed carcasses with a ratio of 1 kg of meat to 1,2 ℓ of water.

Résumé

*PRODUCTION D'UN BOUILLON DE VIANDE DE POULET CONVENANT À LA PRODUCTION EN MASSE D'UN VACCIN D'*HAEMOPHILUS GALLINARUM

*On a préparé du bouillon de viande de poulet selon diverses formules, qu'on a utilisé en quantités de 200 litres pour vérifier leur aptitude à favoriser la croissance d'*Haemophilus gallinarum. *Le meilleur rendement (0,1% de cellules entassées, ou 10^9 unités formatrices de colonies par ml) a été obtenu avec un milieu préparé à partir de carcasses entières dépiautées, à raison d'un kg de viande pour 1,2 l d'eau.*

Matsumoto & Yamamoto (1975) showed that the minimum effective immunizing dose of a *Haemophilus gallinarum* bacterin grown in artificial medium was 10^8 colony-forming units per dose. *H. gallinarum* is, however, a fastidious organism and yields of 10^8 organisms/mℓ are difficult to obtain, particularly in large volumes of media. This problem is further complicated by the fact that, in order to accommodate 2 serotypes of *H. gallinarum* in a 1,0 mℓ dose of bacterin, 0,6 mℓ of which would be oil, a concentration of at least 5×10^8 colony-forming units/mℓ would have to be obtained for each strain to give a final concentration of $1{,}0 \times 10^8$ colony-forming units of each strain per dose.

H. gallinarum is usually grown in complex media containing either serum or fresh blood as a source of nicotinamide adenine dinucleotide (NAD). The NAD is supplied either in the form of plasma from citrated horse blood (Delaplane, Erwin & Stuart, 1936) or in the form of a yeast extract (Delaplane, Erwin & Stuart, 1938). Gregory (1943) obtained good yields of bacteria by using a chicken meat infusion broth supplemented with fresh raw potatoes. A chicken meat infusion broth enriched with 5% chicken serum was used by Kato (1965) and Matsumoto & Yamamoto (1971, 1975). Recently Rimler Schotts & Davis, (1975) described a modified Casman's medium to which NAD had been added for commercial bacterin production.

Preliminary studies (Buys, unpublished data, 1975) indicate that none of these media was suitable for the bulk production of a commercial bacterin, and this led to the development of a culture medium which could yield sufficient organisms in bulk.

For this purpose, frozen, eviscerated broiler carcasses, down-graded on physical appearance, were bought from local poultry abattoirs and stored at −20 °C until they could be processed.

Four basic media were produced from the carcasses by the following manufacturing processes:

1. Carcasses were flayed and were either minced (Medium 1) or kept whole (Medium 2).
2. Unflayed carcasses were either minced (Medium 3) or kept whole (Medium 4).

To 100 kg of the whole or minced carcasses (flayed and unflayed), 150 ℓ of distilled water was added and the carcasses were cooked at 96 °C for 90 min in a double boiler. The infusion broth thus obtained was siphoned off, care being taken to avoid surface fat, and strained through 2 layers of cotton wool.

Media 1 and 3 (from minced carcasses) were so turbid that before any other ingredients were added they were partially clarified by the following method: 50 mℓ of a 20% solution of egg albumen in distilled water was added to each litre of the broth, which was then heated to boiling point, kept boiling for 2 min and then immediately strained through filter paper.

The following ingredients were subsequently added to 200 ℓ of each of the 4 media:

$Na_2HPO_4.12H_2O$	164 g
KH_2PO_4	274 g
NaCl	1 500 g
Peptone (Oxoid)	1 000 g

After ensuring that the ingredients were thoroughly dissolved, the pH of the medium was adjusted to 7,8 with 1N NaOH and clarified through a Carlson* filter fitted with No. 3 pads. The media (200 ℓ) were either pumped into a fermentor and steam sterilized for 2,5 h at 121 °C, or steam sterilized in 30 ℓ quantities in 40 ℓ containers for 2 h at 121 °C and stored at 4°–8 °C for future use in the fermentor.

Chicken serum, to which glucose had been added, was sterilized through a Seitz (EKSE) filter and added aseptically to the cooled, steam-sterilized broths in the fermentors to give a final concentration of 5% serum and 1% glucose. The media were incubated overnight at 37 °C and samples from each streaked onto blood tryptose agar plates (10% horse blood) to determine their sterility.

To produce the bacterin the medium in each fermentor was inoculated with 1–2% by volume of an overnight culture of *H. gallinarum* which had been grown in the same medium. The culture was incubated in the fermentor at 37 °C for 18–24 h, aerated, and then mixed with a stirrer at 200 r.p.m.

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* John C. Carlson, England, U.K.

TABLE 1 Physical appearance and antigen yield of the 5 different media

	Turbidity after boiling carcasses	% Sediment after steam sterilization	Packed cell volume (%) Av. figures	Colony-forming units per mℓ	Carcasses/ water ratio
Flayed minced carcasses (Medium 1)	++++	10–14	0,01	10^7–10^8	1/1,5
Flayed whole carcasses (Medium 2)	+	0–2	0,075	10^9	1/1,5
Unflayed minced carcasses (Medium 3)	++++	10–14	0,01	10^7–10^8	1/1,5
Unflayed whole carcasses (Medium 4)	++	5–7	0,075	10^9	1/1,5
Flayed whole carcasses (Medium 5)	+	0–2	0,1	10^9–10^{10}	1/1,2

Gram-stained smears and cultures on 2 blood tryptose agar plates were prepared from a sample taken from the fermentor to verify the purity of the growth. One of the blood tryptose agar plates was cross-streaked with a feeder culture of *Staphylococcus epidermidis* to ensure growth of *H. gallinarum*. The yield was assayed by centrifuging 10 mℓ quantities of the sample in Hopkins' tubes at 1 000 g for 60 min to determine the packed cell volume.

To determine the number of viable colony-forming units per mℓ, aliquots of 0,01 mℓ of each dilution of the bacterial suspension were placed in separated areas on tryptose agar plates and surrounded with confluent colonies of feeder culture (Page, 1962).

The essential features of the results obtained with the 4 media as well as their yield of bacteria are shown in Table 1.

Despite clarification with egg albumen and filtration through the Carlson filter, a heavy sediment formed after steam sterilization of the media produced from minced carcasses (Media 1 and 3) and the antigen yield was 7–9 × less than that found with Media 2 and 4. In contrast Media 2 and 4 formed less sediment after sterilization and yielded 10^9 organisms/mℓ.

The necessity for clarifying Media 1 and 3 with egg albumen together with the low antigen yield made them totally unsuitable for bacterin production. Although Medium 4 gave a good antigen yield it formed a sediment up to three times higher than Medium 2. Subsequent improvement of Medium 2 led to the development of Medium 5, which gave the best yield.

This medium was an adaptation of the medium prepared from whole flayed carcasses (Medium 2) and the medium developed by Gregory (1943), who used a ratio of 1 kg deboned minced chicken meat to 2 ℓ of water. The calculation of the ratio of meat to water was based on a figure obtained by Joubert, Potgieter & Gregorowski (1976) in South Africa of a 65% yield of meat from broiler carcasses. To obtain Gregory's (1943) ratio the calculated value based on 65% yield would be 1 kg of whole carcass to 1,3 ℓ of water. The ratio, however, was adjusted to 1 kg of meat/1,2 ℓ of water to allow for the loss of skin and of meat in poorly developed carcasses. This led to an increase in the packed cell volume of the organisms from 0,075% to 0,1% and the yield was 10^9–10^{10} viable organisms/mℓ (Table 1).

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