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COMPARATIVE USE OF AMMONIUM SULPHATE FOR PRECIPTTATION OF COLOSTRAL IMMUNOGLOBULINS

By

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SUMMARY

Immunoglobulins (Igs) from the colostrum were successfully precipitated with fertilizer grade ammonium sulphate (NFC)* and the contents were compared with the Igs precipitated by reagent grade ammonium sulphate (E. Merk). There ws no significant difference in total Igs concentration precipitated by either grade ammonium sulphate. Among 10 samples processed, the Igs concentration purified by reagent grade and fertilizer grade ammonium sulphate ranged from 11.75 to 50.00 (20.69 ± 3.88) and 10.50 to 49.75 (20.47 ± 3.87) gms per litre, respectively. The results revealed that the reagent grade ammonium sulphate can be replaced with fertilizer grade ammonium sulphate.

INTRODUCTION

A variety of methods have been employed for the purification of immunoglobulins (Igs) and related proteins, both from colostrum and serum. In all these methods, 4 basic procedures may include electrophoresis (Ristedt, 1980), gel filtration (Vaerman, 1970), DEAE chromatography (Stanworth, 1960) and precipitation (Herbert, 1974). Precipitation is most commonly done either by salts or with alcohols. For successful results in alcoholic precipitation of Igs, strict adherence to specifications of temperature, pH and ionic strength is necessary. In these circumstances, salt precipitation methods have been used under less than ideal conditions because of its simplicity and immediate reactions (Nichol & Deutsch, 1948). Among salts, ammonium sulphate is frequently used for the precipitation of Igs (Logan et al., 1981). Present study reports the precipitation of colostral Igs by saturated solution of fertilizer grade ammonium sulphate (NFC)* and and its comparison with routinely used reagent grade ammonium sulphate (E. Merk).

MATERIAL AND METHODS

One litre colostrum sample, just after parturition, were taken from ten crossbred cows maintained by the Department of Animal breeding and Genetics, University of Agriculture, Faisalabad. The colostrum samples were centrifuged (3000 rpm for 30 minutes at 4°C). The condensed fat in the from of supernatant layer was removed. The casein from fat free colostrum was separated by coagulation with rennin (rennet (Difco) following the methods of Ward et al. (1977). For each ml of the fat free colostrum 0.01 ml (2%) rennin solution was added and mixed. It was incubated at 37°C till curdiling. The curd was broken with glass rod and the whey was separated from clotted casein by centrifugation (2000 rpm for 20 minutes at 4°C).

Separation, Purification and Measurement of Immunoglobulins

Saturated solutions of both ammonium sulphates (NFC and E. Merk) were prepared, separately. The solutions were kept in refrigerator at 4*C overnight. On the following day pH was adjusted to 7.2 with diluted ammonia solution and were filtered through Wattmann-2 filter paper. The Igs i the whey were precipitated with the saturated

* National Fertilizer Corporation.

solutions separately following the method adopted by Herbert (1974). Saturated solution was added to whey samples at the rate of 45 per cent (to each 55 ml of whey, 45ml of saturated solution). It was stirred for 10 minutes at 4°C, centrifuged (300 rpm for 30 minutes at 4°C) and supernatant was discarded. The sediment was dissolved in phosphate buffered saline (PBS), pH 72, to That of the original volume of whey. The dissolved immunoglobulins were again precipitated with saturated solution of ammonium sulphate with final concentration up to 40 per cent. It was stirred and centrifuged as above. The sediment was dissolved in minimum volume of PBS. The ammonium sulphate was removed by dialysis against several changes of PBS unless no ammonium sulphate was detected in PBS using barium chloride test (James, 1983). Total Igs were measured by colorimetric method (Akhtar et al., 1992).

RESULTS AND DISCUSSION

The present studies report the cheapest method for the purification of Igs and related proteins by using fertilizer grader ammonium sulphate. The results (Table 1) revealed no significant difference in total Igs concentration purified by reagent grade ammonium sulphate and by fertilizer gade ammonium sulphate. Among 10 samples processed, the Ig concentration purified by reagent grade ammonium sulphate and fertillizer grade ammonium sulphate ranged from 11.75 to 50.00 (20.69 ± 3.88) and 10.50 to 49.75 (20.47 ± 3.87) gm per litre, respectively. This nonsignificant difference in ig concentration precipitated by either method may be due to the fact that the "salting out" phenomenon depends on the concentration of the salt and molecular characteristics of the protein. Igs even can be precipitated using minimum concentration of ammonium sulphate ranging from 14 to 18 per cent (Lehninger, 1970). So the saturated solution of fertilizer grade ammonium sulphate contained salt required to precipitate the Igs. It may be assumed that a solution not containing desired optimum concentration of the salt will fail to precipitate any protein whereas higher concentration will begin to precipitate non-Ig proteins as well (stone & Gitter, 1969). The variation in individual samples in Ig concentrations is due to the random collection of samples ignoring breed, age, season and calving number (Hodate et al., 1987; Devery-Pocius and Larson, 1983).

Table 1: Concentration of colostral immunoglobulins precipitated by fertilizer grade and reagent grade ammonium sulphate

Sample No.	Ig Conc (g/L)	
	Reagent grade	Fertilizer grade
1	15.54	15.01
2	26.32	26.95
3	50.00	49.75
4	24.35	24.00
5	11.75	10.50
6	14.45	11.90
7	12.38	11.85
8	12.87	12.06
9	15.10	14.90
10	29.21	28.87

To purify Igs from one litre of colostrum, approximately one litre of saturated solution of ammonium sulphate was required. The estimated cost of reagent grade ammonium sulphate is 28 times more than fertilizer grade ammonium sulphate. The results revealthat reagent grade ammonium sulphate can be replaced with fertilizer grade ammonium sulphate for the precipitation of colostral Igs. Further studies are needed to use the fertilizer grade ammonium sulphate for precipitation of igs and related proteins from the serum.

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