IMMUNOLOGICAL GROWTH PROMOTION OF BULLS BY A SYNTHETIC VACCINE INHIBITING THE ENDOGENOUS SOMATOSTATIN.

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Introduction :

Bovine transforms nitrogen into proteins with a poor yield.
The development of growth promoters which increase nitrogen retention at the muscular protein level thus presents a great economical importance. Anabolic agents such as natural or artificial sexual hormones are very efficient to improve meat production. But they are not easily accepted by consumers due to the risk of residues. A recent EEC guiding rule completely banned their use in meat production despite the scientific evidences for the safety of 17 β estradiol, testosterone, progesterone and two synthetic molecules as trenbolone acetate and zeranol.
Taking this attitude into account, new approaches must be proposed to improve meat production. Treatment with somatotropin (GH) or peptidic growth promoters (GRF, IGF) could be of great interest in the future. But for now, they are very expensive to produce and their administration is rather unrealistic without simple delivery systems efficient during several weeks.
Spencer et al. (1)(2) described an elegant method for improving growth in lambs. This method is based on an auto-immunization against somatostatin (somatotropin release inhibiting factor, SRIF). Neutralization of the somatostatin inhibitory effects on growth by antibodies, generated in treated animals, can result in increased muscle development. The peptidic nature of somatostatin allows to avoid the residue problem. But effective adjuvants acceptable in term of public health consideration must be developed before the auto-immunization method will become applicable in growth promoting on farms.
Immunological castration was obtained by Carelli et al.(3) by administering, in an aqueous medium, gonadoliberine (LH-RH) conjugated to the synthetic immunomodulator glycopeptide N-acetylmuramyl-L-alanyl-D-isoglutamine.
Muramyl dipeptide (or tripeptide) derivatives of SRIF were tested as growth promoters in young bulls. We shall present in this paper the effects of this treatment on live weight gain, carcass characteristics and antibody response.
A new homologous bovine GH radioimmunoassay is also described. This assay permitted very sensitive GH measurements in the plasma of animals under experiment. GH content of pituitaries after slaughtering was also determined.

Determination of GH content in the pituitaries
As soon as possible after slaughtering, the skulls were opened, the pituitaries were removed and freezed immediately in dry ice. The following operations were achieved at 4°C. Each freezed hypophysis was cut into small pieces using a scalpel blade. Conjonctive and hard tissues were discarded.
Minced tissue was weighed. The material was transfered quantitatively in an all glass grinder and submitted to successive homogeneizations in 0.12 M phosphate buffer, pH 8.5 containing anti-proteases PMSF and Trasylol (Bayer). The decanted fractions were pooled and their total volume was about 6 ml per hypophysis. These suspensions were agitated overnight in an ice bath. The extracts were centrifuged at 25,000 X g during 20 min. at 4°C (step I). Pellets were extracted again with 2 ml buffer and centrifuged (step II). The pooled supernatants from steps I and II were dialyzed at 4°C against 0.05 M sodium bicarbonate during 24 hr, dialysis bath being changed 3 times. Finally, they were freezed and lyophilized. The lyophilized powder corresponding to individual hypophysis was weighed and a precisely weighed amount of about 100 ug was sampled for bGH determination.
The samples were first diluted at 10 µg/ml of phosphate buffer. Further dilutions were prepared at 1/10, 1/100, 1/1,000 and 1/5,000. One hundrer ul of these dilutions were used for the bGH measurements by RIA.

RESULTS AND DISCUSSION

Variance analysis of the body live weight gain (LWG) has been performed on 27 animals still alive at the end of the 8 months experiment (16 controls and 11 treated animals). Final difference of LWG between control treated animals was 31,3 kg per animal, i.e. 11 % over controls ($p < 0.1$)(Fig. 1). If the performances of killed animals were being taken into additional consideration, the difference in the life weight between control and treated animals would be found to be 34,9 kg per animal, in favour of the treated ones. The deviation measured between the life weight gain of treated animals of batch 5 (SRIF-MTP treated) and 3 (SRIF-MDP treated) was low (5,7 kg) and without any statistical meaning.
During the experimental period, the food consumption of the treated animals was 50 kg higher than that of control animals.
For the same food consumption, the total weight of a treated animal was 30 kg higher than that of control animals.
The food consumption per kg diminished in treated animals from 5.46 (controls) to 5.06 kg (treated ones) representing 400 g less in favor of the treated animals.
In figure 2, temporal evolution of LWG of treated and control animals is compared to plasma titer in anti-SRIF antibodies.
Analysis of the graph shows that the treatment gave moderate results during the first two months, a steeper increase in weight in the following three months, an apparent slow down in the remaining period.
Specific binding was noted 3 months after the initial injection and is restricted to the animals treated with SRIF-MDP or SRIF-MTP.
Nevertheless, specific binding remained low and titers never exceeded one hundred. The method of antibody measurement have to be improved since it was not attempted to determine the optimum conditions (type of tracer, B/F determination, etc...).
Anti-SRIF antibody titers of sera can appear low compared to the observed growth effect. Thus, it seems that a complete neutralization of endogenous somatostatin is not required to provoque a growth enhancement.

Material and methods :

Animals

Thirty young bulls (6 months old around 250 kg each, "bleu-blanc-belge" type) were kept during a two months observation period for adjustment to husbandry conditions and baseline measurements. They were allocated to five treatment groups in order to balance as regards weight. At the beginning of the experiment, each group contained 6 animals.

Immunization protocol

Two groups were injected four times with 100 µg of muramyl di- or tripeptide of SRIF. The animals of 3d and 4th groups were treated with a mixture of equivalent amounts of muramyl di- or tripeptide and SRIF uncoupled. The last group received excipient only.

Samples were solubilized in 9 ‰ sodium chloride supplemented with 40 % polyethyleneglycol 6000. Intramuscular injections in the neck of animals were performed at days 0, 10, 30 and 60 of the 8 months period, each animal receiving 3 ml of solution. During the 8 months treatment period animals were fed at will. Each month, they were weighed and blood samples were taken for further analyses.

Anti-SRIF antibody detection

The detection of free binding sites for SRIF in bull serums has been performed at various periods after initial treatment using ^{125}I Tyr - SRIF as tracer.

A charcoal separation was used for measurement of bound and free radioactivity after a 48 h incubation period, at 4°C, of the tracer in the presence of various dilutions of the serum samples.

Growth hormone assay

Bovine growth hormone was prepared from pituitaries by extraction in 0.3 M potassium chloride, pH 5. Purification to electrophoretical homogeneity was performed using successive chromatographies on CM-Sephadex C-25, Con A-Sepharose 4B, Sephadex G-100 and MONO-Q HR 10/10 as already described for human growth hormone purification (4).

Iodine 125 labeling of the hormone was done using iodogen (5) and the tracer was purified on ACA 54.

Bovine plasma was made free from GH by immunosorption on a column of anti-bGH antibody coupled to Affigel 15 (BioRad Lati).

Radioimmunoassay tubes were incubated in two steps :

1) incubation of plasma samples or standard dilutions of hormone (100 or 200 µl) for 16 h. at 20°C with 100 µl of anti-bGH antibody (≈ 8201 ; dilution 1/4,000) in phosphate buffer (final volume : 400 µl).

2) 100 µl (^{125}I bGH (about 25,000 cpm) were added and the tubes were incubated during 4 H at 20°C. GH-antibody complexes were allowed to precipitate during 30 min in the presence of 1 ml of immobilized anti-rabbit IgG antibody suspension (S.A.P., Techland).

Carcass analysis (costal segment and carcass : relative composition in muscles, adipo-connective tissue and bones) was performed. The treated animals exhibited similar proportions in these compartments as compared to the controls (figure 3, 4, 5).

The following parameters have been measured in animal blood taken monthly throughout the 8 month experiment : blood glucose, thyroid hormones (total T3 and T4), testosterone. They were not found different between treated and control animals.

Table 1 shows GH content of two series of hypophyses taken at the slaughter of the experimental animals.

From these limited results, it appeared that hypophyses from immunized animals had a lower content in GH. This observation could result from a pituitary exhausting in GH.

GH levels in blood samples taken monthly did not differ significantly between control and treated groups (fig. 6).

It is well known that GH secretion is a pulsatile phenomenon. The absence of difference in GH concentration between blood samples taken monthly cannot of course be interprated as a lack of effect of the immunization treatment at the GH level. Nevertheless, the existence of target tissues for somatostatin other that the hypothalamo-hypophysis axis (for example : gut) must be kept in mind to explain mode of action of the vaccine.

Somatomedine (IGF1) has been tentatively measured in blood using a commercial system dedicated to human assay (SMC RIA kit, Nichols). Only two series of animals (one control and one SRIF-MDP treated serie) have been examined. No difference has been observed between these two groups.

Compared to the results obtained by Spencer et al (2) with lambs, we can conclude from our experiment :

1) The synthetic vaccine used in our study was immunogenic per se, without Freund Complete Adjuvant which was a component of Spencer's vaccine. The use of such adjuvant in meat producing animals is questionable from a public health point of view.

2) Spencer et al observed an increase in height, particularly at the level of leg length, beside the weight increase of the treated lambs. It was not the case for bulls treated in our experiment with SRIF-MDP or -MTP : we observed a weight increase but the treated animals were not higher. This observed difference could result from species, ovine versus bovine, or more probably from age, young lambs versus young adult bulls.

3) No effect on basal levels of GH was noted by Spencer et al. Figure 6 of the present paper suggests the same conclusion. In our experiment, we did not attempt to measure GH response to a stimulation by arginine infusion. Under these circumstances, Spencer et al observed a GH release greater in the treated animals.

4) They also found a small, but significant, difference in the levels of bioassayable somatomedine activity between treated and control lambs, the treated lambs having higher somatomedine activity. When assayed with a radioimmunological method used in human endocrinology, we found no difference of IGF 1 levels in sera from control and SRIF-MDP or -MTP treated bulls.

Another experiment was performed using mixed type animals treated under more practicable conditions for bovine meat production : bulls were treated with MDP-SRIF conjugate according to the injection scheme described above, but the animals were sacrified 4 months after the 1st injection. In this particular study, there was no difference in LWG between treated and control group and we did not observed the apparition of anti-SRIF antibodies.
Clearly, further studies are needed to evaluate the efficacy of such growth promoting treatment in function of chemical structure of the synthetic vaccines and their mode of administration. Their mode of action should also be studied on laboratory animals by performing experiments fully controlled in term of endocrine systems and after that significant and systematic immunization has been insured.

Table 1

GH content of two series of hypophyses taken at the slaughter of the experiment animals.

Control		Immunized	
Animal #	mg GH/g hypophysis	Animal #	mg GH/g hypophysis
311	25.3	331	12,9
313	16.9	332	12.6
314	26.4	333	5.1
316	21.1	334	14.3
		336	19.2

References

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(4) Closset, J., Smal, J., Gomez, F. and Hennen, G. (1983). Biochem. J., 214 : 885-892.
(5) Fraker, P.J. and Speck, J.C. (1978). Biochem. Biophys. Res. Commun., 80 : 849-855.

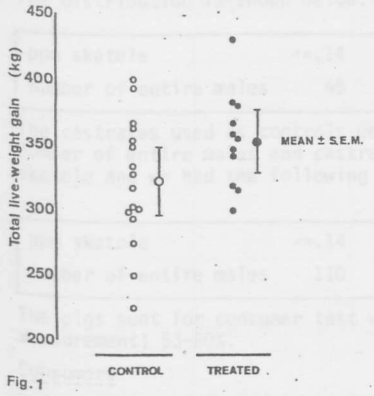

Fig. 1

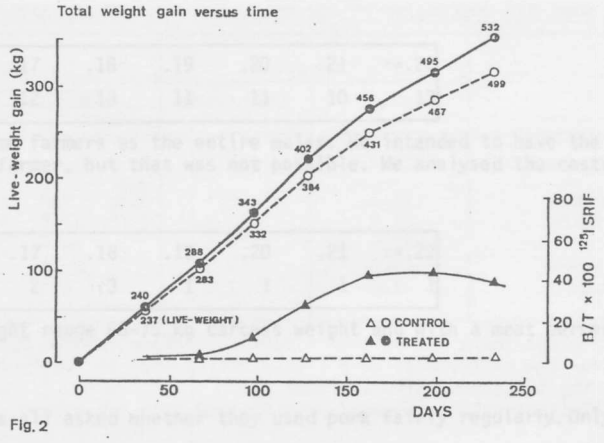

Fig. 2

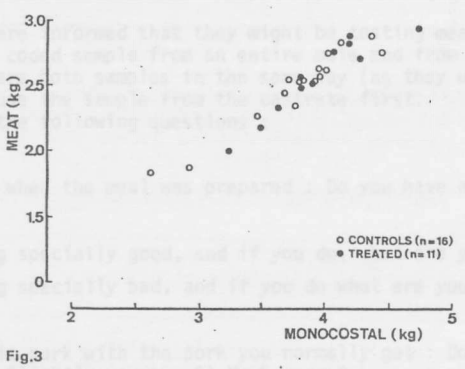

Fig.3

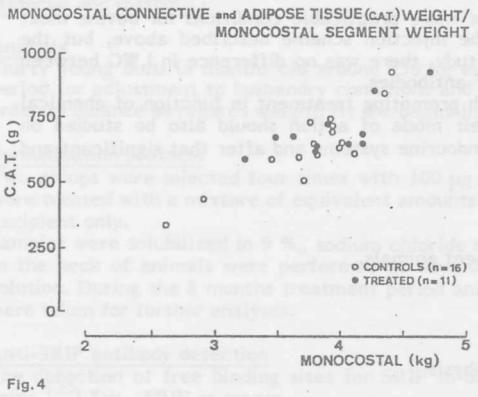

Fig. 4

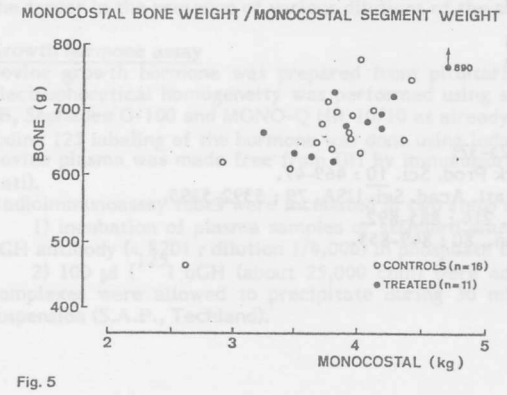

Fig. 5

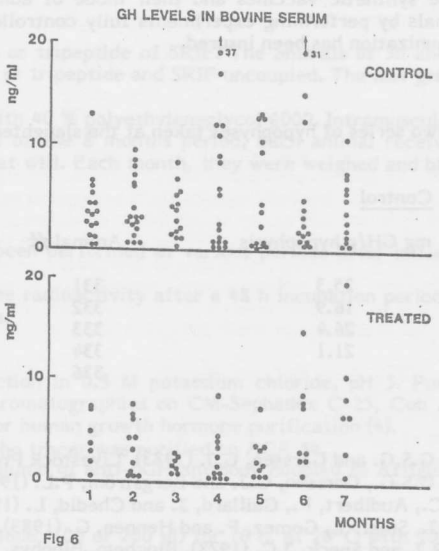

Fig 6