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Original article

Preparation of serocolostrum by membrane microfiltration

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Abstract – Treatment of bovine, equine and caprine colostrum by membrane microfiltration with a pore size of 0.1 μm was studied in order to obtain a specific separation of whey colostrum components. These components are most interesting for imparting passive immunity and positive physiological action to newborn mammals. From the carrying out of 80 kg batches of colostrum, the microfiltration equipment used allowed the recovery of at least 80% of the IgG and other minor whey proteins in the microfiltrate. This liquid, named serocolostrum, is crystal clear, free of blood and somatic cells as well as fat globules and casein micelles, and it has a high hygienic and bacteriological quality (less than 10 CFU·mL^{-1}). Specific concentration of the serocolostrum IgG proteins by high molecular weight cut-off (MWCO: 100 kg·mol^{-1}) membrane ultrafiltration was also studied in order to obtain purified IgG products suitable for preliminary animal trials. Purity (IgG/TS) as high as 90% was obtained. The use of a lower MWCO UF membrane (8 kg·mol^{-1}) allowed concentrations of growth factors (TGF-β and IGF–1) but the observed separation did not agree with the data of the literature, i.e. a binding of IGF–1 with a 45 kg·mol^{-1} protein.

Colostrum / membrane / microfiltration / ultrafiltration / serocolostrum / IgG / TGF-β / IGF-1

Résumé – Préparation de « sérocolostrum » par microfiltration sur membrane. La mise en contact de colostrums de vache, jument et chèvre avec une membrane de microfiltration ayant un diamètre de pores de 0,1 μm permet de réaliser une séparation spécifique des composants du sérum, composants d'intérêt pour obtenir une immunisation passive du jeune mammifère. Avec l'équipement MF pilote utilisé, la mise en œuvre de 80 kg de colostrum conduit à une récupération d'au moins 80 % des IgG et des protéines mineures dans le microfiltrat. Ce liquide, appelé sérocolostrum, est limpide, il ne contient ni cellule sanguine, ni cellule somatique, ni globule gras, ni micelle de caséine. Sa qualité bactériologique est particulièrement élevée (moins de 10 CFU·mL^{-1}). La concentration spécifique des protéines IgG, contenues dans le sérocolostrum par ultrafiltration sur membrane (ayant un pouvoir de coupure élevé, 100 kg·mol^{-1}) a également été étudiée avec pour objectif l'obtention de produits enrichis en IgG (pureté d'au moins 90 %) pouvant être utilisés en expérimentations sur les jeunes animaux. Grâce à l'emploi de membranes UF de plus petit pouvoir de coupure (8 kg·mol^{-1}), la concentration spécifique des facteurs de croissance (TGF-β, IGF-1) a pu être étudiée. La perméation déterminée pour l' IGF-1 n'était pas en accord avec les données de la littérature qui précise que dans le colostrum et dans le lait, ce facteur de croissance serait lié avec une protéine de 45 kg·mol^{-1}.

Colostrum / membrane / microfiltration / ultrafiltration / sérocolostrum / IgG / TGF-β / IGF-1

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1. INTRODUCTION

Colostrum is defined as the mixture of the lacteal secretions produced by the mammary gland during the three days following parturition. Its composition greatly varies during these three days [1, 6]. Total solids, protein, casein, fat and mineral salts, expressed in %, respectively, decrease from 23.9; 14.0; 4.8; 6.7 and 1.11 to 13.6; 4.1; 2.9; 4.3 and 0.81. Lactose increases from 2.7 to 4.7%. It is known for its laxative property [13] due to a thermoresistant component causing fast removal of the "meconium" from the intestinal tracts of the offspring [10].

Because of its high content in immunoglobulins (Ig), mainly (95%) of the subclass IgG_1, specifically concentrated from blood by the mammary cells [21], colostrum was considered as having an essential physiological action for many species (cow, horse and pig). Indeed, it imparts passive immunity to the newborn mammals through a direct passage across the intestinal cells (for a limited period of time: 12–48 h), which is hypothesised to be either specific (horse and pig) or non-specific (cow and goat) [16]. Several studies which were reviewed by Scammell [35] also provide evidence of the efficacy of colostrum in human clinical applications such as treatment of acute infant gastroenteritis [11] and chronic refractory diarrhoea due to Cryptosporidium in AIDS patients [29, 31]. More recently, a lot of attention has been paid to colostrum because of its exceptional content in (i) many growth factors such as EGF (Epithelial Growth Factor), IGF (Insulin Growth Factor), NGF (Nerve Growth Factor) and TGF-β_2 (Transforming Growth Factor), which play a critical role in the regulation and differentiation of a variety of cells [15, 16, 26–28] and (ii) cytokines [10] with particular interest in a proline-rich polypeptide which was shown to improve the outcome of Alzheimer's Disease patients with mild to moderate dementia [18]. Consequently, colostrum and its derivatives may be of a great interest to the cosmeceutical and nutraceutical markets.

Production of colostrum by most healthy cows is in excess of the calf's requirements [6] and consequently, preservation of surplus colostrum for future use has been largely studied. Indeed, the bacteriological quality of colostrum is often very poor (Initial Total Count close to or even higher than 10^6 CFU·mL^{-1} with a high content in coliforms and some pathogens such as *E. coli* and *Salmonella typhimerium*) [6]. Colostrum can also contain some proportion of blood. Attempts to avoid the irreversible degradation of colostrum properties have been reviewed by Foley and Otterby [6]. Heat treatment cannot be envisaged because of the high thermal sensibility of Ig. Controlled acidification either with lactic starters or with mineral acid was proposed, but it leads to coagulation of the casein. Chemical food additives (sodium benzoate or benzoic acid) and even formaldehyde have been proposed, but their use at farm level is risky, and efficiency as well as acceptability by the calves were not satisfactory. Immediate freezing at –20 °C is considered to be the best means to prevent nutrient breakdown during storage. However, microbial contaminants are still present in the frozen product and the thawing temperature and time may allow undesirable fermentation to occur. Bactofugation of skimmed colostrum was also proposed [39, 40] but it is well known that this technology reduces the bacterial count by only 95%, which is largely insufficient. Ionisation of colostrum for feeding calves has been studied and it is officially authorised, at a maximal dose of 10 kGy [14], but as it is well known, it is likely that ionisation induces undesirable flavour compounds from milkfat breakdown and it also leaves inside the treated colostrum dead microbial cells with most of their enzymatic activities. More recently, Mortensen [25] has proposed using the same process, i.e. membrane (pore size 1.4 μm) microfiltration of skimmed colostrum, as the one applied to removing contaminating bacteria from skim milk but no indication was given in this patent on the decimal reduction of the total count observed in the MF colostrum.

In spite of the supposed immunoprotective effect of colostrum leukocytes [12, 36] and a higher and different glycosylation of casein GMP (glycomacropeptide) which could lead to a closer similarity with human GMP [2], in a first approach, it can be concluded that most of the interesting components are in the whey part of the colostrum. Considering, on the other hand, the potential of membrane microfiltration for the selective separation of milk particles [5, 24, 32], application of this technology to colostrum as already patented by Garcin et Paviot [7] appears to be an elegant way to prepare, in mild conditions of time and temperature, a high quality "serocolostrum" without blood red cells, somatic cells, fat globules, bacteria and casein micelles of which all the whey proteins and mainly the IgG could easily be specifically concentrated by membrane ultrafiltration. This paper deals with the studies and the results obtained by our team with bovine, caprine and equine colostrums.

2. MATERIALS AND METHODS

2.1. Colostrums

Amounts of 18 to 80 kg of pooled frozen bovine, caprine or equine colostrum were thawed at a maximal temperature of 30–40 °C in jacketed tanks and diluted by 50% by adding either RO water containing KCl 0.03 $mol \cdot L^{-1}$ and 0.02 $mol \cdot L^{-1}$ NaCl or milk ultrafiltrate, and then separated on "Elecrem" equipment (Elecrem, Aubervilliers, France). The separated cream which represented 10 to 15% (v/v), was discarded or, in order to improve the yield in skimmed colostrum, diluted with the aforementioned saline solution and re-submitted to separation.

2.2. Membrane microfiltrations

The skimmed diluted colostrums were maintained at 33 °C–37 °C for 20 min in order to reach stabilisation of the milk saline equilibrium. Then, they were introduced and maintained at temperature lower than 40 °C in microfiltration equipment (Tetra Pak Processing SNC, Le Blanc Mesnil, France) comprising (i) according to the volume of colostrum to be treated, between 0.2 and 4.6 m^2 of "Membralox" membrane (Pall Exekia, Bazet, France), with an average pore size of 0.1 μm and (ii) a recirculation loop of the microfiltrate in order to obtain a Uniform Transmembrane Pressure (UTP) [32, 34]. According to the experiments, continuous diafiltration (as the same rate as the MF flux) with 8 to 10 diavolumes of the aforementioned saline solution was realised on the diluted colostrums (dilution rates ranging from 0.50 to 0.88) in order to yield a maximum amount of IgG in the microfiltrate.

2.3. Membrane ultrafiltrations

Proteins contained in the microfiltrates were then concentrated (Volumic Concentration Factor or VCF ranging from 15 to 30) in ultrafiltration equipment comprising either 9.7 m^2 of spiral wound polymeric membrane with a cut-off of 5 $kg \cdot mol^{-1}$ (T.I.A., Bollène, France), or 6.8 m^2 M1 or M5 ceramic membrane (Rhodia Orelis, St Maurice de Beynost, France) with, respectively, a cut-off of 100 $kg \cdot mol^{-1}$ and 10 $kg \cdot mol^{-1}$ and finally, a 6.65 m^2 piece of equipment equipped with a "sunflower" Tami ceramic membrane (Nyons, France) with a cut-off of 8 $kg \cdot mol^{-1}$.

2.4. Freeze-drying

Serocolostrum fractions and UF retentates were freeze-dried in CIRP CS 10-0.8 equipment (SGD Serail, Argenteuil, France) and then kept at room temperature until the animal experiments, the carrying out of which, and the analytical measurements used, have already been described by Grongnet et al. [8] for the feeding of calves, Chavatte-Palmer et al. [3] and Clément et al. [4] for the feeding of newborn foals and by Le Huërou-Luron et al. [17] and Marion et al. [22] for the feeding of piglets.

Table I. Microfiltrate composition ($g \cdot kg^{-1}$).

	TS	TNM	IgG
Bovine	9.6–23.7	3.8–11.6	2.2–5.0
Caprine	5.5–10.1	3.2–6.2	2.1–5.3
Equine	10.5–11.7	3.8–4.1	1.8–2.5

TS: Total Solids; TNM: Total Nitrogen Matter (N × 6.38); IgG: Immunoglobulin G.

2.5. Analysis

Total solids, fat, proteins and lactose were determined by using reference analytical methods previously described by Saboya et al. [33].

Immunoglobulins (IgG) were determined as described by Mancini [20] or by RP-HPLC on a Waters 600 E system (Waters S.A., St Quentin en Yvelines, France) equipped with a PLRP-S 1,000 A 150 × 4.6 mm column (Touzart et Matignon, Vitry sur Seine, France). Elution was done by using HPLC-grade acetonitrile (ACN) (Carlo Erba, Nanterre, France) containing 0.1% trifluoroacetic acid (TFA) in gradient conditions as follows: 35 to 38% ACN for 8 min and increase ACN concentration to 43% in 10 min. Detection was carried out at 280 nm. Standards were bovine IgG (Sigma-Aldrich Chimie, St Quentin Fallavier, France) and equine IgG (gift from Prof. Grongnet).

TGF-β contents were determined by sandwich enzyme immunoassay (Quantikine, R & D systems, Abingdon, UK).

IGF-1 contents were determined according to Louveau et al. [19].

3. RESULTS AND DISCUSSION

3.1. Microfiltration experiments

Whatever the origin of the colostrum (bovine, equine or goat), permeation fluxes around 25 $L \cdot m^{-2} \cdot h^{-1}$ were obtained with a UTP varying between 40 and 50 kPa. Such fluxes are at least 3 times less than those observed when "ideal whey" is separated from milk [25, 37, 38], probably because of the lower temperature used (37 °C instead of 50 °C) and the high content (ranging from 38.2 to 13.6 $g \cdot kg^{-1}$) in large-sized IgG protein molecules. The composition of the crystal clear (slightly yellow-pink coloured) microfiltrate ("serocolostrum") varied according to the protein content of the treated colostrum and the rate of dilution (0.50 to 0.88). The range of variation of TS, TNM and IgG contents in this diluted serocolostrum fraction for the three studied species is summarised in Table I. As expected, the proportion of IgG in the TNM largely varies according to the original colostrum composition linked to the number of mixed post-partum milkings and the rate of dilution used before skimming and for diafiltration. Instantaneous MF rejection coefficients for IgG were determined with caprine 1st and 2nd milking colostrums. They increased similarly versus time as expected (impoverishment of their content in the MF retentate) from 0.46 to 0.89 and from 0.52 to 0.87, respectively. No bacteria was enumerated in all of the obtained "serocolostrums", whatever the initial count (between 10^6 and 10^7 $CFU \cdot mL^{-1}$), and samples were kept at room temperature for one year without any growth [7].

3.2. Ultrafiltration experiments

The parameters used in the ultrafiltration experiments are summarised in Table II. Average fluxes, as is very well-known, were varying according to the temperature used, the protein content of the treated "serocolostrum", the molecular cut-off of the UF membrane utilised and the VCF between 12 and 53 $L \cdot m^{-2} \cdot h^{-1}$. Table III summarises the range of determined composition of the obtained ultrafiltrates and retentates. For bovine

Table II. Operating conditions for ultrafiltration of colostrum microfiltrate (pooled with diafiltrate).

	Type of membrane	Temperature (°C)	Transmembrane pressure 10^{-5} Pa	Flux ($L \cdot h^{-1} \cdot m^{-2}$)
Bovine	100 000 $g \cdot mol^{-1}$	20–41	0.8–2.8	12–32
	0.02 µm	19–27	0.6–1.1	18–53
	8 000 $g \cdot mol^{-1}$	35–50	2.8–2.9	27–43
Caprine				
1st milking	5 000 $g \cdot mol^{-1}$	21–22	0.5–3.2	18.6–19.6
2nd milking	5 000 $g \cdot mol^{-1}$	21–26	1.2–1.9	17–31
Equine	10 000 $g \cdot mol^{-1}$	30–35	3–3.5	25
	5 000 $g \cdot mol^{-1}$	21–31	85–2.3	13–33

Table III. Retentate and ultrafiltrate composition.

		Type of membrane ($g \cdot mol^{-1}$)	TS ($g \cdot kg^{-1}$)	TNM ($g \cdot kg^{-1}$)	IgG ($g \cdot kg^{-1}$)
Bovine	UF	100 000	8.4–8.6	1.1–1.4	0.4–0.6
	Ret.		94–112	89–105	65–84
	UF	8 000	53.8	1.8	
	Ret.		121.9	60.9	
Caprine	UF	5 000	n.d.	n.d.	0.05[1]
			"	"	0.03[2]
	Ret.	5 000	109[1]	108[1]	92[1]
			48[2]	47[2]	35[2]
Equine	UF	5 000	5.4	0.23	0.01
		10 000	11.6	0.51	0.09
	Ret.	5 000	42.2	24.7	13.0
		10 000	53–78	50–76	30–49

[1] First milking; [2] second milking; n.d. = not determined.
TS: Total Solids; TNM: Total Nitrogen Matter (N × 6.38); IgG: Immunoglobulin G.

serocolostrum, use of a 100 $kg \cdot mol^{-1}$ MWCO membrane, carried out in order to remove not only lactose and soluble mineral salts but also low MW whey proteins, allowed us to reach an enriched IgG retentate with ratios of TNM/TS close to 0.95 and IgG/TNM varying between 0.56 and 0.80. Similar purifications were obtained with caprine and equine serocolostrums. On the other hand, the results obtained with caprine 1st and 2nd milking colostrum, of which the IgG/TNM ratios are, respectively, 0.56 and 0.41, show that the use of the same UF parameters and the same UF equipment allows to obtain a more IgG-enriched UF retentate (Tab. IV) when the starting material has a higher IgG content. The initial relative ratio between the two milkings, which is 0.56/0.41 = 1.366, is kept in the final resulting freeze-dried UF retentates (Tab. IV).

3.3. Recovery of IgG, TGF-β and IGF-1 in the proposed process

In order to prepare large quantities of purified IgG for animal experiments, 4 successive complete trials, each with between

Table IV. Freeze-dried powders' composition (g·100 g).

	TS	TNM	IgG
Bovine	96–99	80–93	51–73
Caprine			
1st milking	99	97	85
2nd milking	99	94	58
Equine	98	91	55

TS: Total Solids; TNM: Total Nitrogen Matter (N × 6.38); IgG: Immunoglobulin G.

80 and 90 kg of bovine colostrum obtained by the mixture of the 2nd, 3rd and 4th milking were realised. The amounts and average compositions of the products obtained at each step of the process, from an 80 kg batch of colostrum, are indicated in Figure 1.

Because the volume of colostrum cream represented about 10% of the whole colostrum, recovery of IgG in skimmed colostrum was found to be, as expected, around 90%. This yield can be increased by cancelling cream separation and directly submitting the diluted whole colostrum to MF with an extended diafiltration step. Losses of IgG during the MF step originated by the lost retentate (6.7 to 5.0% of the volume used) and the dead volume of the MF equipment used were found to be in the range of 20 to 33%. Losses in the UF step only resulted from the dead volume of the equipment and reached about 10 to 15%. Finally, in our experiments, the global recovery of IgG was at the maximum 64%. Such a recovery can be improved when larger quantities of colostrum are used, seeing that losses due to the dead volumes of the MF and UF equipment are fixed, whatever the volume of treated liquid.

The content in TGF-β of the diluted skimmed colostrum was 100.7 ng·mL^{-1} i.e. 1.47 μg per g of TNM or 3.03 μg per g of IgG. This determination agrees with those of Pakkanen [26]. The content in the UF retentate was 160.0 ng·mL^{-1} i.e. 1.80 μg per g of TNM or 1.95 μg per g of IgG, that showed a loss in this component during the UF step in the ultrafiltrate in spite of the fact that growth factor should be present in dairy products as a dimer of 2 × 12 720 g·mol^{-1} [23].

The content in IGF-1 of the diluted skimmed colostrum was 206 ng·mL^{-1} i.e. 3 μg per g of TNM or 6.2 μg per g of IgG. The determined content in UF retentate was 200.0 ng·mL^{-1} i.e. 2.25 μg per g of TNM or 2.44 μg per g of IgG. That also confirmed the high loss in the ultrafiltrate which was determined as 5.3 ng·mL^{-1} on average, as shown in Figure 1. Such a loss in serocolostrum ultrafiltrate with a 8 kg·mol^{-1} UF membrane agrees with the MW of IGF-1 (7 649 g·mol^{-1} according to Rindernecht and Humbel [30] and Frankenne et al. [9]) but does not agree with the results of Vega et al. [38] who claimed that in colostrum, most of the IGF-1 is bound with a 45 kg·mol^{-1} protein.

3.4. Bacteriological quality of the products

In agreement with the literature, all the colostrums were highly contaminated with a Total Count higher than 10^6 CFU·mL^{-1} and 10^4 CFU·mL^{-1} coliforms. No more than 1 to 2 mesophilic CFU·mL^{-1} were enumerated in the serocolostrums obtained through MF with a 0.1 μm pore sized membrane. But, because of the difficulty of satisfactory sanitation of spiral wound UF equipment, UF retentates as their resulting freeze-dried powders have a slightly increased mesophilic TC (around 10^2 CFU·mL^{-1}) but no enumerable coliform.

4. CONCLUSION

Treatment at mild temperatures (37–40 °C) of whole colostrum by membrane microfiltration using a pore size of 0.1 μm and the hydraulic concept of uniform transmembrane pressure (UTP) or an equivalent device allows to obtain a serocolostrum free of blood and somatic cells and with a high bacteriological quality (less than 10 CFU·mL^{-1}). The IgG as well as the other minor components contained in this "serocolostrum", which can be obtained with a satisfactory yield if an extended diafiltration

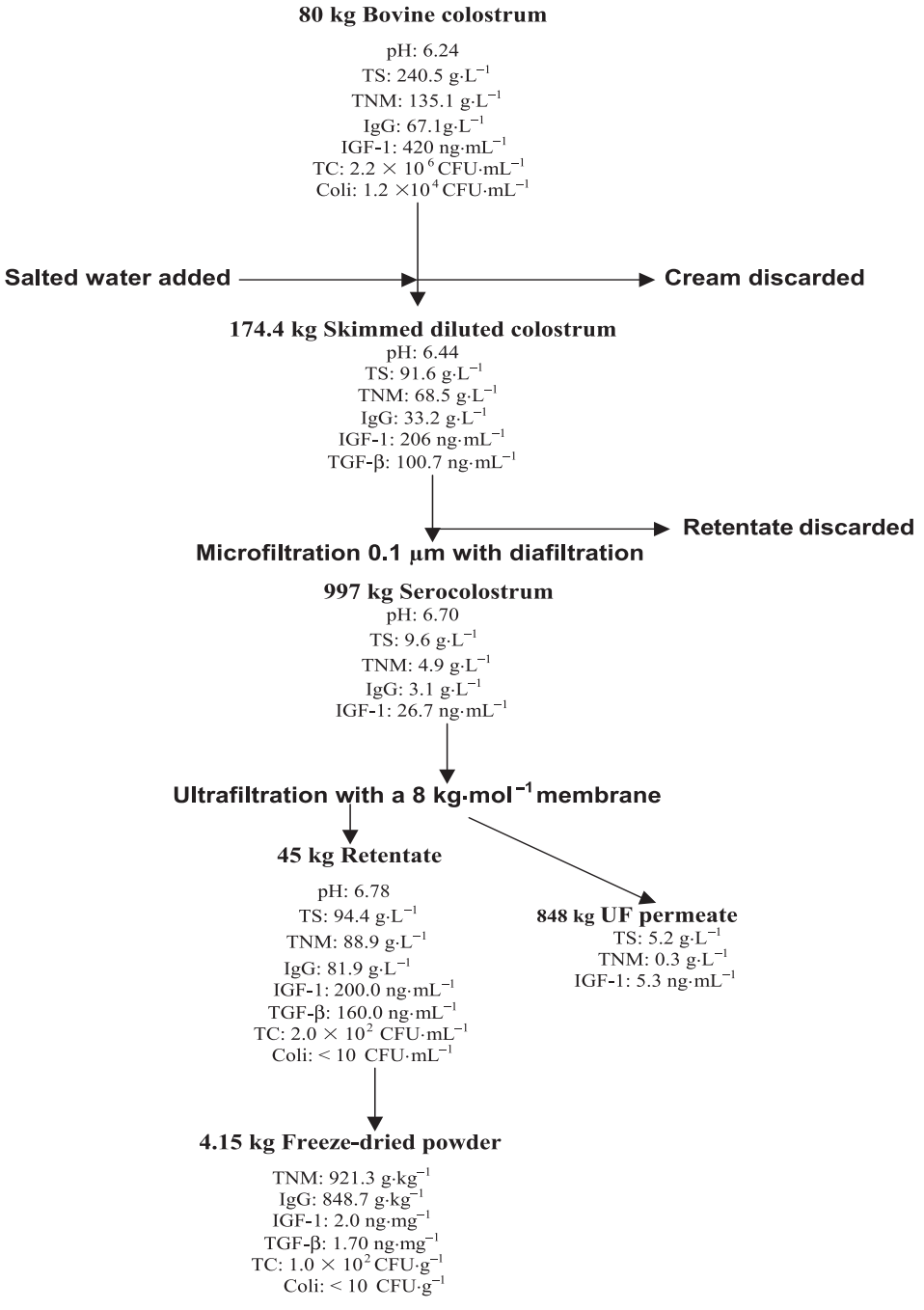

Figure 1. Separation of serocolostrum components by membrane processes.

step is carried out, can be used directly for feeding young animals or concentrated by membrane UF (membranes with a molecular cut-off of 5 kg·mol^{-1} appear to be the most appropriate for a maximum retention of growth factors) in order to obtain purified products. The preliminary animal trials done with the freeze-dried powders of IgG obtained in our study showed that they kept their physiological properties.

Indeed, as mentioned above, the bovine freeze-dried UF retentates obtained from this study were given by le Huërou-Luron et al. [17] and Marion et al. [22] to newly weaned piglets. Compared with the control group, a highly interesting increase in both duodenal villous height of 22% and protein synthesis of 21% in the duodenum was seen [22]. The obtained results are in favour of an improved gut health around weaning in piglets [17]

Equine frozen and freeze-dried UF retentates made according to the developed process were used by Chavatte-Palmer et al. [3] and by Clément et al. [4] for the feeding of newborn foals. Absorption of IgG as well as the foals' immunisation measured by the IgG plasma level were found to be identical to those of foals receiving high quality equine colostrum [3, 4].

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