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**Original article** 

# Effects of a bovine colostrum-supplemented diet on some gut parameters in weaned piglets<sup>\*,\*\*</sup>

Antoine HUGUET, Bernard Sève, Jean LE DIVIDICH, Isabelle LE HUËROU-LURON\*\*\*

Unité Mixte de Recherches, Systèmes d'Élevage, Nutrition Animale et Humaine, INRA, Domaine de la Prise, 35590 St-Gilles, France

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**Abstract** – The present study investigated the effects of a bovine colostrum-supplemented diet on gut post-weaning adaptation and health in piglets. Thirty-six 21-d-old piglets were allocated to one of the three following dietary treatments: sow-reared (SR), weaned on a control starter diet (WCtrl) or on a starter diet supplemented with bovine colostrum (WCol) until slaughter at 28 d or 35 d of age. Gastric pH and intestinal bacteriological, structural and functional parameters were determined. Compared to WCtrl, the gastric pH was lower (P < 0.05) and the duodenal lactobacilli:coliform ratio was higher (P = 0.05) in WCol piglets. The relative small intestine weight was 18% (P < 0.05) higher in WCol piglets than in SR piglets. Duodenal villous height was lower (P < 0.01) in WCtrl than in SR piglets, whereas the value for WCol piglets was intermediate. The weaning-increased crypt cell proliferation was not affected by bovine colostrum supplementation. The mucosal ribosomal capacity was higher (P < 0.05) in W than in SR piglets. In conclusion, a diet supplemented with colostrum induced, although not always significantly, variations of gut parameters, suggesting that globally, colostrum may limit weaning-induced gut structural and microbial alterations. The observed effects occurred early and were maintained throughout the post-weaning adaptive phase.

bovine colostrum / intestine / microflora / mucosa growth / weaning

# **1. INTRODUCTION**

From a nutritional aspect, weaning of piglets is characterised by a temporary anorexia inducing a growth check that is associated with a decline of sanitary status. The ban within the European Union of growth promoting antibiotic-supplemented diets in livestock production requires finding alternative issues. One alternative is the

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<sup>\*\*</sup> Corresponding author: Isabelle.Luron@rennes.inra.fr

supplementation of the weaning diet with natural substances. However, large discrepancies in the reported results exist.

Bovine colostrum is a commercially available co-product of the dairy industry. Bovine colostrum-supplemented starter diet improves the growth performance and sanitary status of piglets during the early postweaning period [1, 2]. These beneficial effects could be explained by both an observed increase in feed intake level as early as the first days after weaning [1] and a likely direct effect on gut health. Giving bovine colostrum orally prevents chemically-induced villous atrophy in mice [3] and stimulates mucosal healing of patients suffering from inflammatory gut disease [4]. It reduces diarrhoeal symptoms in infants [5] and protects intestinal mucosa from pathogen bacteria adhesion [6].

Altogether, these studies suggest that bovine colostrum could be an alternative to growth promoting antibiotics in order to prevent weaning-induced gut disorders. The aim of our study was to understand how bovine colostrum interacts with the porcine gut at weaning. Therefore, the effects of a bovine colostrum-supplemented diet on the structure and digestive functions of the intestinal mucosa, as well as on the composition of luminal microflora of piglets during the first two post-weaning weeks were investigated.

# 2. MATERIALS AND METHODS

### 2.1. Animals and dietary treatments

The experiments were conducted under the guidelines of the French Ministry of Agriculture for animal research. Thirty-six crossbred Pietrain × (Large White × Landrace) 21 d-old piglets from the experimental herd of INRA (Saint-Gilles) were allocated into 12 littermate triplets on the basis of BW. Within each triplet, piglets were randomly allocated to one of the following three dietary treatments: sow-reared (SR), weaned on a control starter diet (WCtrl) or on a starter diet supplemented with freeze-dried defatted bovine colostrum at the rate of 5% of dry matter (DM) (WCol). After weaning of WCtrl and WCol piglets, the remaining piglets of littermates were adjusted to 10 piglets with additional non-experimental piglets. Six littermate triplets were slaughtered at 28 d of age, and the 6 other littermate triplets were slaughtered at 35 d of age. No creep feed was provided during the suckling period. At 21 d of age, a catheter was surgically implanted into a jugular vein of piglets after 2-h fasting. The catheter was introduced in a bag placed on the dorsal side of the piglet's neck and fixed with a surgical tape. Sow-reared piglets were returned to their mothers after a 3-h recovery, whereas weaned (W) piglets were individually housed in stainless steel metabolic cages providing visual contact with each other. Room temperature was initially set at 32 °C, progressively decreased to 28 °C on d 7 post-weaning and was maintained constant thereafter.

In the present study, sow-reared piglets were considered as a reference of piglets with undisturbed digestive functions compared to piglets that had to face weaninginduced dietary changes. Weaned piglets were fed a complete formula based on cereals, soybean meal, maltodextrin, dairy products, fish meal and vegetal oil (Tab. I). Bovine colostrum was individually collected from the first three milkings of healthy cows from the experimental herd of INRA (Méjussaume) and frozen at -20 °C. After thawing, pooled bovine colostrum was defatted using a dairy separator and freeze-dried. Total protein content of the freeze-dried bovine colostrum was 550 mg·g<sup>-1</sup> DM, IgG representing 41% of total protein. The various growth factors present in the freezedried bovine colostrum were incompletely defined but included insulin (368  $ng \cdot g^{-1}$ DM), IGF-I (1.61  $\mu$ g·g<sup>-1</sup> DM), and transforming growth factor  $\beta$  (670 ng·g<sup>-1</sup> DM). The two starter diets were formulated to meet W piglet requirements [7] and were similar in net energy content (19 and 18.8 MJ·kg<sup>-1</sup> DM for WCtrl and WCol diets, respectively). The WCtrl diet was

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**Table I.** Composition of the diets given to weaned piglets.

	Diet		
	WCtrl <sup>1</sup>	WCol <sup>2</sup>	
	g·kg <sup>−1</sup> air-	dried diet	
Ingredients			
Wheat seeds, ground	246.6	246.6	
Barley seeds, ground	247	247	
Soybean meal	160	160	
Maltodextrins	42	52	
Dehydrated whey	150	90	
Freeze-dried defatted	-	50	
bovine colostrum			
Soluble fish protein	82.5	82.5	
concentrate			
Sunflower oil	28	28	
Trace element and vitamin	5	5	
premix <sup>3</sup>			
CaCO <sub>3</sub>	15.2	15.2	
$CaHPO_4 \cdot 2H_2O$	20	20	
L-lysine-HCl	1.25	1.25	
DL-methionine	0.85	0.85	
L-threonine	1.3	1.3	
L-tryptophan	0.3	0.3	
	MJ·kg <sup>-1</sup> dry matte		
Crude energy	19	18.8	

<sup>1</sup> WCtrl, control starter diet.

<sup>2</sup> WCol, bovine colostrum-supplemented starter diet. <sup>3</sup> Contains the following amount of vitamins and minerals in g·kg<sup>-1</sup> of diet: calcium carbonate (excipient) 550.69; zinc oxide (78% Zn) 25.7; copper sulphate (25% Cu) 16.0; manganese oxide (62% Mn) 11.7; iron carbonate (40% Fe) 50.0; calcium iodate (62% I) 0.32; cobalt sulphate (21% Co) 1.9; sodium selenite (1% Se) 6.0; retinol (500,000 IU·g<sup>-1</sup>) 4.8; vitamin A/vitamin D-3 (500,000/100,000 IU·g<sup>-1</sup>) 1.2; cholecalciferol (500,000 IU·g<sup>-1</sup>) 0.96; tocopherol (500 IU·g<sup>-1</sup>) 16.0; menadione (22.7%) 1.76; thiamin (98%) 0.4; riboflavin (80%) 2.5; niacin (pure) 6.0; Ca pantothenate (99%) 3.0; pyridoxine (pure) 2.0; biotin (2%) 2.0; folic acid (pure) 0.4; cyanocobalamin (0.1%) 10.0; ascorbic acid (pure) 20.0; choline chloride (60%) 266.67.

free of any growth promoting additive. The WCol diet contained solely freeze-dried bovine colostrum in replacement of dehydrated whey and maltodextrin. Weaned piglets were fed twice a day (at 0900 and 1700 h) and had free access to water. The actual feed intake level was measured by weighing the feeding trough before and after meals. Weaned piglets were pair-fed until the end of the experimental period. More precisely, the WCtrl diet was offered for 60 min to the WCtrl piglets. Then WCol piglets were offered the same amount of feed intake level as WCtrl piglets within triplets, but unexpectedly, the feed intake of WCol piglets was lower than that of WCtrl piglets. The daily metabolisable energy (ME) intake level of W piglets is shown in Figure 1. The daily ME intake level of SR piglets is estimated according to Le Dividich and Sève [8] and varied from 7646 ± 1368 kJ·d<sup>-1</sup> at d 29 to 5355  $\pm$  1350 kJ·d<sup>-1</sup> at d 35.

#### 2.2. Slaughter procedure

Prior to slaughter, all piglets were fasted for 3 h. Two hours before slaughter, the piglets were given intraperitonealy a solution of bromodeoxyuridine (Sigma-Aldrich Chimie, Lyon, France) at the rate of  $50 \text{ mg} \cdot \text{kg}^{-1}$ BW. Fifteen minutes before slaughter a solution of [<sup>15</sup>N] valine was given via the jugular catheter in 28d-old piglets for the determination of the fractional protein synthesis rate (FSR). Piglets were killed through intracardiacal injection of an overdose of sodium thiopental  $(30 \text{ mg} \cdot \text{kg}^{-1})$ BW; Nesdonal, Merial, France) and exsanguinated. Fresh gastric contents were immediately collected and the pH was determined using a pH meter (704 model, Metrohm, France). The entire small intestine (SI) from the pyloric sphincter to the ileocaecal junction was gently removed. The SI was laid on a glass sheet placed on crushed ice. From the first 50 cm (duodenum), two 2-cm long segments with their contents (1 and 20 cm from the pyloric sphincter) were removed, weighed, transported in Ringer buffer and stored at 4 °C until bacteriological analyses. The remaining SI was then flushed with cold isotonic saline, blotted dry and weighed. Thereafter, two duodenal 2-cm long segments were fixed in phosphate-buffered formalin (10%, pH 7.6) for 48 h at 4 °C and then stored in

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ethanol:water (3/1, vol/vol) solution at 4 °C until structural analyses and paraffin-embedding for immunohistochemistry analyses, whereas the mucosa of a duodenal 30-cm long segment was scraped, frozen in liquid nitrogen and stored at -80 °C until protein content, enzyme activity, and bound and free pool [<sup>15</sup>N] valine analyses. Additionally for the latter analyses, mucosa of a 10-cm long segment of the ileum (60 cm before the ileocaecal junction) was scraped, frozen in liquid nitrogen and stored at -80 °C.

# 2.3. Bacteriological analyses

Duodenal contents of W piglets were collected in order to evaluate the antibacterial efficacy of the bovine colostrum-supplemented diet. The contents were serially diluted in sterile saline and then cultured on Columbia with horse blood (dilutions  $10^{-3}$ to  $10^{-6}$ ), McConkey (dilutions  $10^{-3}$  to  $10^{-6}$ ), and Rogosa (dilutions  $10^{-3}$  to  $10^{-6}$ ) agar base plates that were incubated at 37 °C for 48, 24 and 48 h, respectively, for aerobia, coliform and lactobacilli counts. The colony forming units were counted on each plate and for each bacterial type on both duodenal sites as described by Krueger et al. [9]. The values were divided by the mass of duodenal contents collected and expressed on a logarithmic basis (log N). The ratio of coliforms:aerobes, lactobacilli:aerobes and lactobacilli:coliforms were finally calculated.

#### 2.4. Structural analyses

Measurements of villous height and crypt depth were made under blind condi-

Figure 1. Age related evolution of daily metabolisable energy (ME) intake in weaned piglets fed the control starter diet (WCtrl) ( $\blacksquare$ ) or the bovine colostrum-supplemented starter diet (WCol) ( $\blacktriangle$ ). Values are means  $\pm$  SD.

tions. Samples were micro-dissected under binocular optical. According to the technique of Goodlad et al. [10], villi and crypt sizes were measured as reported [11].

#### 2.5. Immunohistochemistry on bromodeoxyuridine-labelled cells

The samples of small intestine were cut longitudinally, dehydrated in ethanol (70% and 95% successively), put in butanol, and embedded in paraffin. Serial, histologic sections of 5 µm thickness were stained immunohistochemically using a protocol adapted from the Bromodeoxyuridine Immunohistochemistry System kit (Oncogene, San Diego, USA). Briefly, the sections were maintained in a moist chamber at 37 °C and digested with a trypsin (concentrate:diluant 1/1; vol/vol) solution, incubated first with an anti-bromodeoxyuridine antibody, and then during 30 min with the diaminobenzidin mixture. The cells were scored as positive if they contained unequivocal brown diaminobenzidin deposit. No quantitative threshold was employed. The measurements on 20 crypts per sample were performed using a light microscope (Eclipse E400, Nikon) and analysed by an image analyser (Lucia software) coupled with the light microscope via a camera (Digital camera DXM1200, Nikon). The proportion of proliferating crypt cells was quantified by counting the number of bromodeoxyuridinelabelled cells and expressed as the percent of the total number of cells per crypt. The migration index was defined as the distance between the cell located in the bottom of the crypt and the labelled cell that had migrated

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the furthest along the crypt-villous axis as described by Wynford-Thomas and Williams [12].

# 2.6. Enzyme activity assays

The protein content in mucosal homogenates, lactase (EC 3.2.1.23), maltase (EC 3.2.1.20), and aminopeptidase N (ApN, EC 3.4.11.2) activities were measured according to Marion et al. [13]. The resulting enzymatic units were expressed as nmol of substrate hydrolysed per min (IU).

# 2.7. Fractional protein synthesis rate measurements

The FSR measurements were performed according to Sève et al. [14]. Briefly, a solution of  $[^{15}N]$  valine was prepared by mixing 20% of  $[^{15}N]$  valine 99% mol percent excess (Tracer Technologies, Sommerville, MA) with 80% of unlabelled L-valine (Sigma-Aldrich Chimie, Lyon, France) to get 19.8 mol percent excess final enrichment and by diluting with water to give a final concentration of 0.15 mol· $L^{-1}$ . This solution was injected at a dose of 1.05 mmol·kg<sup>-1</sup> BW. Blood was sampled 7 min before, and 7 and 14 min after the injection (t0) of [15N] valine in order to determine the decrease in plasma [<sup>15</sup>N] valine enrichment. Free and protein-bound valine enrichments were measured by GC-MS coupling (VG Platform II GC 8000, Fisons Instruments, Altricham, Great Britain) and GC-combustion-isotope ratio MS coupling (Isochrom GC, Fisons Instruments, Altricham, Great Britain), respectively. FSR was expressed in percent per  $d(\% \cdot d^{-1})$ . Mucosal protein and RNA concentrations were measured as reported by Sève et al. [14].

#### 2.8. Hormone assays

Plasma insulin and IGF-I concentrations were determined in duplicates using validated RIA [15,16]. The samples were run in a single assay. Plasma insulin and IGF-I concentrations were measured once a day (at 1000 h) at 21, 22, 25, 27 and 34 d of age. For insulin, the intra-assay CV was 2.5% at 1.6 ng·mL<sup>-1</sup>, and the average sensitivity of the assay, defined as 90% of total binding, was 0.114 ng·mL<sup>-1</sup>. Plasma IGF-I concentrations were determined after an acid-eth-anol extraction according to Louveau and Bonneau [16]. The intra-assay CV was 11% at 48.7 ng·mL<sup>-1</sup>, and the average sensitivity of the assay, defined as 90% of total binding, was 0.31 ng·mL<sup>-1</sup>.

#### **2.9. Statistical analyses**

Analysis of variance was performed using the GLM procedure of the Statistical Analyses System [17]. For growth, structural, digestive functions, and microbiological parameters the effect of age using the residual variation between triplets as error, and the effect of dietary treatment and age × dietary treatment interaction were tested. For protein synthesis parameters, the effect of dietary treatment using the residual variation within triplets as error, and the effect of site and site × dietary treatment interaction were tested. For hormone assays, the effect of dietary treatment using the residual variation within triplets as error, and the effect of age and age × dietary treatment interaction were tested. The regression procedure of the Statistical Analyses System was used to assess relationships between villous height and ME intake level. The effect of dietary treatment on villous height with ME intake level as a covariate was tested according to the GLM procedure. When an effect was significant (P < 0.05), adjusted Least Squares Means (Ismeans) were compared (t test). The values presented are lsmeans  $\pm$  SEM. The differences were declared significant at P < 0.05 and tendencies at P < 0.1 were noticed.

#### **3. RESULTS**

#### 3.1. General

The mean BW of the 21d-old piglets was  $6.3 \pm 0.7$  kg. BW was significantly

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**Figure 2**. Age related evolution of gastric pH in sow-reared piglets (SR) (opened bars) and weaned piglets fed the control starter diet (WCtrl) (closed bars) or the bovine colostrum-supplemented starter diet (WCol) (hatched bars). Values are lsmeans  $\pm$  SEM. Lsmeans with superscripts without a common letter differ (P < 0.05).

**Table II.** Bacterial counts in the duodenum of weaned piglets fed the control starter diet (WCtrl) or the bovine colostrum-supplemented starter diet (WCol)<sup>1, 2</sup>.

Dietary treatment	WCtrl	WCol	SEM	P value
Coliform (log N coliform/log N aerobia)	0.74	0.68	0.03	P = 0.20
Lactobacilli (log N lactobacilli/log N aerobia)	0.83	0.84	0.02	P = 0.80
Lactobacilli:coliform ratio	1.19 <sup>a</sup>	1.63 <sup>b</sup>	0.16	P = 005

<sup>1</sup> Values are lsmeans. Lsmeans without a common superscript within a row differ (P < 0.05).

 $^{2}$  Values of bacterial counts were expressed in log of counts per g of contents (log N).

increased with age (P = 0.006) and affected by dietary treatment (P < 0.001). At 28 d of age, the BW of SR piglets  $(9.4 \pm 0.4 \text{ kg})$  was 36% and 32% higher than in WCtrl (6.9  $\pm$ 0.4 kg) and WCol  $(7.1 \pm 0.4 \text{ kg})$  piglets, respectively. At 35 d of age BW of SR piglets was 10% and 24% higher than in WCtrl  $(9.9 \pm 0.4 \text{ kg})$  and WCol  $(8.8 \pm 0.4 \text{ kg})$  piglets, respectively. Although both groups of W piglets were pair-fed, feed intake of WCol piglets was slightly, but non significantly (P > 0.05) lower during the second post-weaning week, explaining their lower BW. No diarrhoea was observed during the experimental period. There was no effect of age on any of the parameters studied, except for plasma insulin concentrations. There was no age × dietary treatment interaction, except for gastric pH and plasma IGF-I concentrations. Therefore, when age and age  $\times$ dietary treatment interaction were non significant, data from the two slaughter ages were pooled.

# 3.2. Influence of dietary treatment on gastric pH and duodenal bacterial counts

A significant age × dietary treatment interaction (P = 0.002) was found for gastric pH. At 28 d of age, gastric pH in WCol piglets was the lowest and different (P <0.05) from values in SR and WCtrl piglets that tended to be different (P = 0.07) (Fig. 2). At 35 d of age, gastric pH similar in both SR and WCol groups was lower (P < 0.05) than that in the WCtrl group. Bacteriological analyses were performed on W piglets only. There was no effect of the dietary treatment on coliform and lactobacilli counts expressed per total aerobia microflora (Tab. II), whereas the lactobacilli:coliform ratio was 37% higher (P = 0.05) in WCol piglets compared to WCtrl piglets.

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Table III. Duodenal villous height, crypt depth, crypt cell proliferation and migration index in sowreared piglets (SR) and weaned piglets fed the control starter diet (WCtrl) or the bovine colostrumsupplemented starter diet (WCol)<sup>1</sup>.

Dietary treatment	SR	WCtrl	WCol	SEM	P value	
Mucosal structure						
Villous height $(\mu m)^2$	521 <sup>a</sup>	437 <sup>b</sup>	472 <sup>ab</sup>	20	P < 0.01	
Crypt depth (µm)	314	323	338	12	P = 0.37	
Villous height crypt depth ratio	1.7 <sup>a</sup>	1.3 <sup>b</sup>	1.3 <sup>b</sup>	0.1	P < 0.01	
Crypt cell BrdU labelling <sup>3</sup>						
Proliferation (% SR piglets)	100	116	115	5	P = 0.08	
Migration index (µm)	306	364	387	36	P = 0.31	

<sup>1</sup> Values are lsmeans. Lsmeans without a common superscript within a row differ (P < 0.05).

<sup>2</sup> Adjusted lsmeans according to covariance analyses using ME intake level during the 3 days preceding

slaughter as covariate. <sup>3</sup> Two hours before slaughter, a solution of bromodeoxyUridine (BrdU) was intraperitonealy injected (50 mg kg<sup>-1</sup> BW). The proportion of BrdU-labelled crypt cells per total crypt cells was expressed as a percent of the proportion observed in SR piglets (proliferation). The migration index was defined as the distance between the cell located in the bottom crypt and the labelled cell that has migrated the furthest along the crypt-villous axis.

# **3.3. Influence of dietary treatment** on duodenal structure, crypt cell proliferation and migration index

The relative SI weight was 10% (P = 0.15) and 18% (P < 0.05) higher in WCtrl  $(33.5 \pm 1.4 \text{ g} \cdot \text{kg}^{-1} \text{ BW})$  and WCol  $(35.9 \pm$ 1.4  $g \cdot kg^{-1}$  BW) piglets, respectively, than in SR piglets  $(30.4 \pm 1.5 \text{ g} \cdot \text{kg}^{-1} \text{ BW})$ . There was no difference between the W piglets. Since the regression procedure was significant between villous height and ME intake level, the effect of dietary treatment on villous height was tested with ME intake level as a covariate. Villous height was 16% reduced (P < 0.01) in WCtrl piglets compared to SR piglets, whereas the value for WCol piglets was intermediate and did not differ significantly from the values of the other dietary treatments (Tab. III). There was no effect of dietary treatment on crypt depth. The villous height/crypt depth ratio was similar in both W groups and 24% lower (P < 0.005) than that in SR piglets. Crypt cell proliferation in W piglets was 15% higher (P < 0.05) than that in SR piglets. There was no dietary treatment effect on migration index.

# 3.4. Influence of dietary treatment on enzyme activities and protein synthesis

There was no effect of dietary treatment on protein contents (average value: 113 ± 1.5 mg $\cdot$ g<sup>-1</sup> duodenal mucosa). Duodenal lactase and aminopeptidase N, but not maltase, specific activities (SA) were significantly affected (P < 0.05) by the dietary treatment (Tab. IV). Compared to SR piglets, lactase SA was 39% reduced (P < 0.05) in WCtrl piglets. The 29% reduction (P =0.08) in WCol piglets was intermediate. Aminopeptidase N SA was similar in both W groups and 24% (P < 0.05) lower than in SR piglets.

Mucosal protein synthesis was determined in the duodenum and ileum on 28dold piglets only. There was no significant site  $\times$  dietary treatment interaction (P = 0.17). The ribosomal capacity was similar in W piglets and 21% higher (P < 0.01) than in SR piglets (Tab. V). Dietary treatment tended (P = 0.07) to affect FSR that was higher in W piglets compared to SR piglets. Ribosomal activity was similar across the

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Table IV. Digestive enzyme activities in the duodenum of sow-reared piglets (SR) and weaned piglets fed the control starter diet (WCtrl) or the bovine colostrum-supplemented starter diet (WCol)<sup>1</sup>.

Dietary treatment	SR	WCtrl	WCol	SEM	P value
Lactase (IU·mg <sup>-1</sup> protein) <sup>2</sup>	31 <sup>a</sup>	19 <sup>b</sup>	22 <sup>ab</sup>	3	P = 0.04
Maltase (IU·mg <sup>-1</sup> protein)	120	120	143	15	P = 0.46
Aminopeptidase (IU·mg <sup>-1</sup> protein)	74 <sup>a</sup>	58 <sup>b</sup>	54 <sup>b</sup>	5	P = 0.03

<sup>1</sup> Values are lsmeans. Lsmeans without a common superscript within a row differ (P < 0.05).

<sup>2</sup> IU: nmol of substrate hydrolysed per min.

Table V. Duodenal and ileal protein synthesis in 28d-old sow-reared piglets (SR) and weaned piglets fed the control starter diet (WCtrl) or the bovine colostrum-supplemented starter diet (Col)<sup>1, 2</sup>.

	Dietary treatment			Site					
	SR	WCtrl	WCol	SEM	P value	duodenum	ileum	SEM	P value
Ribosomal capacity (μg RNA·mg <sup>-1</sup> protein)	36 <sup>a</sup>	43 <sup>b</sup>	44 <sup>b</sup>	1	P < 0.01	36 <sup>a</sup>	47 <sup>b</sup>	2	<i>P</i> < 0.001
Fractional protein synthesis rate (%·d <sup>-1</sup> )	99	120	125	8	P = 0.07	120	109	7	P = 0.260
Ribosomal activity (mg protein/mg RNA/d)	28	27	29	21	P = 0.67	33 <sup>a</sup>	23 <sup>b</sup>	1	<i>P</i> < 0.001

<sup>1</sup> Values are lsmeans. Lsmeans without a common superscript within a row (dietary treatment or site) differ (P < 0.05).

<sup>2</sup> Fifteen minutes before slaughter, a solution of [<sup>15</sup>N] valine (19.8 mol percent excess final enrichment and 0.15 mol· $L^{-1}$  final concentration) was administered via the jugular catheter in 28d-old piglets (1.05 mmol· $kg^{-1}$  BW). Free and protein-bound value enrichments were measured. Fractional protein synthesis rate was calculated as the ratio of protein bound to protein-free valine enrichments and expressed in percent per d.

dietary treatments. Compared to the duodenum, the ribosomal capacity was 30% higher (P < 0.001) and the ribosomal activity was 30% lower (P < 0.0005) in the ileum. FSR was not significantly different in the duodenum and ileum.

# 3.5. Influence of dietary treatment on plasma insulin and IGF-I concentrations

A significant age × dietary treatment interaction was found (P = 0.004) on plasma IGF-I concentrations (Fig. 3A). Compared to SR piglets, plasma IGF-I concentrations were 70% (P < 0.01) and 63% (P < 0.05) lower in W piglets on d 22 and 27 respectively, whereas there was no significant effect of dietary treatment on d 35.

There was no effect of dietary treatment and age × dietary treatment interaction on plasma insulin concentrations, but a significant effect of age was found (P < 0.001) (Fig. 3B). Compared to d 21, the plasma insulin concentration was increased by 200% (P < 0.001) at d 25 and remained unchanged thereafter.

# 4. DISCUSSION

Weaning-induced modifications of structure, digestive functions and microflora of the gut are well-described in piglets [18-22]. In the present study, the variations of these parameters in W piglets compared to SR piglets were largely in agreement with the literature data. Following the short acute

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Figure 3. Age related evolution of plasma IGF-I (A) and insulin (B) concentrations in sow-reared piglets (SR) ( $\diamond$ ) and weaned piglets fed the control starter diet (WCtrl) ( $\blacksquare$ ) or the bovine colostrumsupplemented starter diet (WCol) ( $\blacktriangle$ ). Values are lsmeans ± SEM. For IGF-I (significant age × dietary treatment interaction) lsmeans with superscripts without a common letter differ (P < 0.05). Insulin superscripts without a common letter indicate a significant age effect (P < 0.05).

phase observed during the first 3-5 postweaning days, the adaptive phase corresponding to the progressive restoration and maturation of the gut and its microflora lasts up to two weeks [20, 23-25]. In the present study, W piglets were slaughtered during this adaptive phase. The similar treatmentinduced effects measured at both ages suggest that observed effects of the colostrum-supplemented diet were maintained throughout this adaptive phase. Therefore the discussion concentrates on the effects of the added freeze-dried colostrum in the weaning diet.

The colostrum-supplemented diet significantly improved the lactobacilli:coliform ratio in W piglets. This effect was mainly caused by numerically lower coliform counts whereas lactobacilli counts were identical between WCtrl and WCol piglets. Lower gastric pH in WCol piglets strengthened the effects of colostral antibacterial components. The coliform population, which may concern some pathogenic strains usually considered as harmful for gut health, was shown to be less resistant to low pH than the lactobacilli population [26]. The relationship between gastric pH and colostrum supplementation remains nevertheless to be explained. A successful use of colostrum in the treatment of diarrhoea caused by enteropathogenic Escherichia coli, a member of the coliform-type microflora, has already been reported in human patients [27-29]. Colostrum contains a large number of antimicrobial components, including lactoferrin, lysozyme, lactoperoxidase which have been shown to inhibit the growth of several pathogenic bacteria. In addition bovine colostral

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immunoglobulins may provide local protection within the gut as described in newborn piglets [30] and may stimulate the SI immune system [5].

The effect of colostrum supplementation was studied within groups of weaned piglets. Moreover, sow-reared piglets were considered as a reference of undisturbed animals considering digestive functions. Therefore suckling piglets were compared with weaned piglets fed a colostrum-supplemented diet in order to evaluate colostrum-induced improvement of weaning transition from digestive physiological aspects. Adding colostrum in the starter diet of weaned piglets resulted in numerically higher SI relative weight and duodenal villous height, indicating that colostrum may stimulate mucosa growth as it has already been demonstrated in rodents [3]. Authors have also described a colostrum-induced enhancement of cell proliferation and migration using in vitro human colonic carcinoma cells and rat SI epithelial cell models of wound repair [3]. Our results in vivo did not confirm these latter observations since any significant variation in crypt cell proliferation and migration was explained by the colostrum treatment. The amount of colostrum reaching the epithelial barrier of the SI in vivo was probably lower than that applied to cells in vitro. Moreover it is well known that weaning-induced crypt cell proliferation is plateauing as early as the third day post-weaning [22, 31]. The possibility that the colostrum-supplemented diet stimulated cell proliferation during the first 3 post-weaning days cannot be excluded.

Weaning-induced changes in the phenotype of enterocytes correspond to the maturation of digestive functions in response to the change from maternal milk to a weaning diet. In agreement with reported data [32– 34], this period is characterised by a decrease in lactase and peptidase specific activities and an increase in maltase specific activity. An additional effect of the colostrum-supplemented diet was not observed, although values of disaccharidases were numerically higher in colostrum-fed piglets. To our knowledge, no previous study documented the effect of colostrum on SI enzyme activities in W piglets.

In agreement with the data of Sève et al. [35], the ribosomal capacity was enhanced by weaning. The addition of colostrum in the weaning diet did not increase FSR. In contrast, in early weaned piglets fed a starter diet supplemented with a bovine colostral fraction high in growth factors for 5 days, duodenal FSR was significantly increased by 20% [36]. The lower amount of growth factors provided by the colostrum in the present study compared to a study of Marion et al. (illustrated by a 4-fold lower amount of colostral IGF-I intake the day before slaughter and no variation in plasma IGF-I levels) could explain the observed discrepancy in the intestinal mucosa response. Hence, a minimal provision of both nutrients and growth factors must be achieved at the epithelial barrier surface to induce change in FSR. The decrease in the ribosomal activity from the proximal to distal SI may be related to the progressively lower input of nutrients toward the distal intestine. In artificially fed neonatal piglets, FSR was twice as high in the jejunum as in the ileum [37].

In conclusion, a diet supplemented with colostrum induced, although not always significantly, variations of gut parameters, suggesting that globally colostrum may limit weaning-induced gut structural and microbial alterations. The present data also suggest that the observed effects of colostrum occurred early and were maintained throughout the first two weeks following weaning, corresponding to the post-weaning adaptive phase. The identification of molecules accounting for these effects will require further investigations.

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