# Endocrine pancreas development at weaning in goat kids

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**ABSTRACT** – Eighteen three-day old Saanen goat kids were divided into MILK and WEAN groups. MILK kids received goat milk to age 48 days; WEAN kids were initially fed milk but started weaning at 25 days and were completely weaned by 40 days. Total intake per group was recorded daily. On day 25, 40 and 48, body weights were recorded, and plasma samples were taken and analyzed for glucose, free amino-acids and insulin. On day 48, all animals were slaughtered and pancreas samples were analyzed for total DNA and RNA content. Histological sections of pancreas were examined by light microscope and images analyzed by dedicated software. Seven days after the beginning of the weaning program, dry matter intake in the WEAN group began to decrease compared to the MILK one. Nonetheless, body weight did not differ throughout the study period. Weaning significantly decreased plasma levels of glucose, amino-acids and insulin. No difference was observed in pancreatic DNA and RNA content. Histological analysis of pancreas showed that the size of pancreatic islets was not different, but islet number per section was lower in the pancreas of WEAN animals. In conclusion, weaning affects glucose and amino-acid metabolism and influences endocrine pancreas activity and morphology.

*Key words:* Pancreas, Weaning, Goat kids.

**Introduction** – Weaning is the stage of life in which milk is progressively substituted by solid feeds. Along this process, the diet shifts from a mixture of casein, lactose and triglycerides to a more complex nutrient mixture. This change requires adaptation of digestive (Kinouchi *et al.*, 2000) and endocrine (Hoet *et al.*, 2000) functions. Weaning is a crucial period in the life of young animals, since it can influence growth, maturation of the reproductive tract and even the quality of the end products (Owens *et al.*, 1993). Furthermore, the adaptation to new dietary conditions may be stressful, as usually the pancreas is not fully functional at weaning (Stoffers, 2004). The aim of the present study was to evaluate endocrine pancreas development around weaning in goats, to obtain information on physiological and morphological changes that occur during the adaptation to the new diet.

**Material and methods** – Three days after birth, 18 male Saanen goat kids were assigned to one of two groups of equal average body weight, MILK and WEAN. Each animal was fed colostrum from its mother for the first 3 days. The MILK group then received goat milk *ad libitum* throughout the experimental period (to age 48 days). The WEAN group received goat milk *ad libitum* to 24 days and then underwent weaning. Weaning mixture was offered *ad libitum* to WEAN kids from 18 to 48 days of age. Starting from age 25 days milk was gradually reduced and it was completely withdrawn from WEAN diet on the 40$^{th}$ day. All animals were fed twice a day (9:00 am and 7:00 pm). The weaning feed mixture consisted of dehydrated alfalfa hay (39.0%), steam-flaked maize (14.5%), soybean whole flakes (9.0%), wheat straw (6.0%), barley grain flakes (5.0%), dried sugar beet pulp (5.0%), maize gluten meal (4.0%), soybean meal (4.0%), sunflower seeds (4.0%), sugar cane molasses (3.5%) and mineral/vitamin supplement (6.0%). Data on goat milk and weaning mixture compositions are shown in Table 1. During the study period, total consumption per group was recorded daily.

Table 1. Experimental feeds' composition.

| | % as fed | %on dry matter |
|---|---|---|
| | Goat milk | |
| Dry matter | 12.7 | |
| Protein | 3.35 | 26.4 |
| Fat | 4.29 | 33.8 |
| Lactose | 4.78 | 37.6 |
| | Weaning mixture | |
| Dry matter | 89.3 | |
| Crude protein | 17.6 | 19.7 |
| Ether extract | 4.74 | 5.30 |
| Starch | 15.9 | 17.8 |

Table 2. Plasma metabolites and insulin level in goat kids.

| | Group | Days of age | | | SEM |
|---|---|---|---|---|---|
| | | 25 | 40 | 48 | |
| Glucose | WEAN | 5.74 | 4.72** | 4.26** | 0.11 |
| (mM) | MILK | 5.93 | 5.53** | 5.55** | |
| FAA | WEAN | 4.73 | 3.88** | 4.03** | 0.18 |
| (mM) | MILK | 4.86 | 5.69** | 5.23** | |
| Insulin | WEAN | 142 | 47.8** | 48.4** | 30.1 |
| (pM) | MILK | 148 | 314** | 441** | |

**=P<0.05; **=P<0.01.*

At the beginning of weaning (day 25), at the end of weaning (day 40) and 8 days after (48 days), individual body weights were recorded and blood samples were taken before the first meal of the day. Plasma was analyzed for glucose (Giesse Diagnostics Srl) and free amino-acids (FAA) (Goodwin, 1968) by spectrophotometric methods, and insulin (Insik-5, Dia Sorin Spa) by radio-immune assay. All animals at 48 days of age were slaughtered five hours after the first meal of the day. Pancreas samples were taken and analyzed for DNA and RNA content (Munro and Fleck, 1966). Fresh specimens of pancreas were fixed in a 10% formalin neutral solution for one week and then washed in alcoholic solutions and embedded in paraffin. Blocks were sectioned (3 µm thickness) and stained with hematoxylin-eosin. Pancreas sections were examined by light microscope and histological images were analyzed with dedicated software (ImageJ 1.38x). The area occupied by the pancreatic islets of Langerhans was then calculated as a percentage of the total area of the section. Data were evaluated by ANOVA using the GLM procedure of SAS (SAS Institute Inc.).

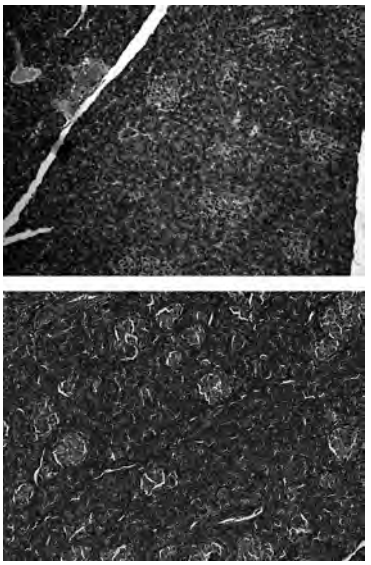
**Results and conclusions** – As long as all the kids were fed milk *ad libitum*, dry matter intake (DMI), expressed as mean per group, was similar (Figure 1). Eight days after the start of weaning (at 33 days of age), DMI in the WEAN group began to decrease (P<0.01) compared to the MILK one (Figure 1). Initially this could be due to refusal of new diet by the WEAN animals. Subsequently, the difference could have been the consequence of an increasing contribution of ruminal activity to the nutritional requirements of the animals (Berg *et al.*, 2005). Nevertheless body weight did not differ between the groups throughout the study period (data not shown), possibly because of a greater development of the digestive tract in WEAN kids (Berg *et al.*, 2005). Goat kids are not ruminant at birth and for the first 7 to 14 days of life the animal's forestomachs are poorly developed, but as dry feeds begin to be consumed, the rumen and the reticulum rapidly increase in size (Berg *et al.*, 2005). Moreover, feed in the rumen could have inflated WEAN kids' body weight, as dry rations are retained in the rumen for several hours for microbial fermentation (Faverdin, 1999). At the completion of weaning (day 40), glucose and FAA began to be significantly lower in WEAN group and the difference was permanent (Table 2). These findings can be considered typical of the transition from

Figure 1. Mean dry matter intake per group.

pre-ruminant to ruminant state. In calves, plasma glucose decreases during the transition to ruminant metabolism (Young *et al.*, 1970). The lower FAA in WEAN kids could have been due to the reduction in DMI, but it is also important to consider that ruminal bacteria create a biomass that passes out of the rumen and is digested in the abomasus and the small intestine and provides a source of amino-acids for the adult ruminant (Berg *et al.*, 2005). As WEAN kids were in the early ruminant state, it is possible that the rumen was not fully functional and unable to provide sufficient protein and energy.

On day 40 of life, also insulin began to be significantly lower in the WEAN than MILK group (Table 2). The difference may have been related to the lower plasma glucose, but the effect could have been enforced by the intake of milk-borne insulin by means of the diet in MILK kids. In fact, insulin is naturally present in goat milk (Magistrelli *et al.*, 2008). Moreover, in suckling rat it has been demonstrated that insulin added to milk replacer increases plasma level of insulin (Kinouchi *et al.*, 2000). Anyway, it is unclear whether milk-borne insulin can cross the mucosa and enter the systemic circulation of suckling kids in a long-lasting bioactive form (Odle *et al.*, 1996). No difference has been observed in pancreatic DNA or RNA content (data not shown), neither in RNA/DNA ratio (data not shown) between the groups, suggesting no difference in the synthetic potential of the pancreas. Analysis of pancreas sections showed that the size of pancreatic islets of Langerhans was not different between groups, but islets were twice less abundant in the pancreas of WEAN animals (5.54% *vs.* 10.9% of the total area, for WEAN and MILK kids, respectively, SEM=1.19, P<0.01) (Figure 2). This last finding may explain the difference observed in plasma insulin level. On the other hand, the lower plasma insulin level could be the cause for the reduced number of pancreatic islets in WEAN kids. Recently, in fact, insulin has been demonstrated to stimulate β-cell proliferation, in rats (Beith *et al.*, 2008). In conclusion, weaning deeply affects glucose and amino-acid metabolism and influences endocrine pancreas activity and morphology. Further studies will verify whether insulin stimulates β-cell proliferation in ruminants.

Figure 2. Histological sections of pancreas of a WEAN (up) and a MILK (down) kid.



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