

## Morphology of small intestinal mucosa and intestinal weight change with metabolic type of cattle

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**ABSTRACT:** The objective of this study was to investigate rumen fermentation, apparent digestibility of nutrients, and morphology of ruminal and intestinal mucosa in two cattle breeds of different metabolic type. From each breed six purebred German Holstein (H) bulls representing the secretion type and six Charolais (CH) bulls representing the accretion type were raised and fattened under identical conditions with *semi ad libitum* feeding of a high energy diet. The animals were used for a digestion trial started at nine months of age and animals were slaughtered at 18 months of age. Body weight (668 vs. 764 kg,  $P = 0.011$ ), body weight gain (1 223 vs. 1 385 g/day,  $P = 0.043$ ), and body protein gain (93 vs. 128 g/day,  $P = 0.001$ ) were lower in H compared to CH bulls. Protein expense per kg protein accretion was higher in H bulls (13.8 vs. 10.2,  $P = 0.001$ ). No significant differences were found in concentration and pattern of ruminal short chain fatty acid and in apparent digestibility of organic matter, crude fibre, and N-free extracts. There were no significant differences in all morphometric traits of rumen mucosa between both cattle breeds. Compared to H, the villi of CH bulls were higher in duodenum (586 vs. 495  $\mu\text{m}$ ,  $P = 0.001$ ) and proximal jejunum (598 vs. 518  $\mu\text{m}$ ,  $P < 0.001$ ), the crypt were deeper in duodenum (295 vs. 358,  $P < 0.001$ ) and proximal jejunum (292 vs. 344  $\mu\text{m}$ ,  $P = 0.020$ ). In contrast, the villi in ileum were higher in H (522 vs. 471  $\mu\text{m}$ ,  $P = 0.006$ ). The weight of total small intestine, as percentage of total body weight, was 1.1 in H and 0.8 in CH ( $P = 0.002$ ). The utilization of food crude protein was positively related to the duodenal ( $P = 0.001$ ) and proximal jejunal villus height ( $P = 0.003$ ) and to the duodenal crypt depth ( $P < 0.001$ ) and negatively related to weight of small intestine ( $P = 0.004$ ). It is concluded, that the higher growth potential and feed efficiency in CH bulls compared to H bulls is not caused by differences in digestion processes, but in size of small intestine, and morphology of small intestinal mucosa. Obviously the duodenum and proximal jejunum of CH bulls adapt to increase the absorptive surface due to the increase in nutrient demand.

**Keywords:** cattle; German Holstein; Charolais; rumen fermentation; nutrient digestibility; ruminal and intestinal mucosa morphology

Cattle of beef breeds growth faster and utilize nutrients better than cattle of dairy breeds as demonstrated recently by Pfuhl et al. (2007) for Charolais (CH) and German Holstein (H) bulls. Energy requirement of CH bulls for maintenance is less than that of H bulls as found by Chudy (2001) in respiration chambers under thermoneutral, energy

deficient conditions. Causes of these differences are not completely clear. The difference in nutrient partitioning towards a higher protein accretion in CH in comparison to H can be related to the lower levels of insulin, glucagon, leptin, and IGF-1 in CH bulls as found before on bulls aged about nine months (Bellmann et al., 2004). Ren et al. (2002)

showed that leptin mRNA levels in subcutaneous and perirenal fat depots, but not in the omental fat depot, were significantly higher in H than in CH. Lipoprotein lipase mRNA expression in the perirenal fat depot of H was greater in abundance than that of Charolais.

Less is known about physiological differences in digestive tract between beef and dairy cattle. It cannot be excluded, that the process of digestion is different between the metabolic types of cattle as found in Holstein, Highland, and Galloway bulls (Voigt et al., 2000). Furthermore, the ruminal and/or intestinal tissue can be different and therefore affect the inflow of absorbed nutrients into intermediary organs. The metabolic activity of these tissues is considerable. The portal-drained viscera (PDV; including gastrointestinal tract, pancreas, spleen and associated adipose tissue) account for about only 3 to 6% of total body mass but their proportion on total energy consumption and whole-body protein turnover accounts for 20 to 35% (Cant et al., 1996; Reeds et al., 1999). The pivotal role of gastrointestinal tissues in both whole-body nutrient demand and systemic tissue supply was discussed in some recent papers (Schaeffer et al., 2003; Drackley et al., 2006). Gastrointestinal tissues are also critical components of the post-absorptive system as they mediate absorption of nutrients and play a role in regulation of metabolite availability to all other tissues in the body.

In intensively reared cattle receiving higher amounts of concentrates not only the absorption surface of rumen papillae but also the height of duodenal and jejunal villi were seen to increase. This fact was confirmed by the positive correlation between the morphometric parameters of ruminal and intestinal mucosa (Zitnan et al., 2003).

Mir et al. (1997) considered the length of villi and depth of crypts and mucosal carbohydrase activity as an important factor in nutrient absorption. The authors found differences in these parameters between different cattle breeds.

We hypothesize that morphology of gastrointestinal mucosa reflects the difference in nutrient utilization between growing cattle of beef and dairy breeds. The objective of this study was to investigate in addition to the ruminal fermentation and the digestibility of nutrients together with the morphology of the gastrointestinal mucosa in two metabolic types of cattle. For this purpose, H bulls representing the dairy type and CH bulls representing the beef type were used.

## MATERIAL AND METHODS

### Animals and nutrition

The used animals were part of the segregating families herd at the Research Institute for the Biology of Farm Animals (see Kuhn et al., 2002). Six purebred CH and H bulls were used, respectively after weaning at four months of age. The animals were housed in a tethering system and fed *semi ad libitum* individually with a diet consisting of concentrates and hay (Table 1) until 18 months of age. Feed refusals were recorded daily and offered feed was adjusted weekly in relation to maintenance requirement ( $530 \text{ kJ ME/kg BW}^{-0.75}/\text{day}$ ) and maximal body weight (BW) gain (GfE, 1995). The bulls were weighed monthly. The experiment was carried out in two periods at nine and 18 month of age. During the digestive trial in period I at nine month of age the animals were fed restrictive.

### Sampling and analyses

**Period I (digestion trial).** The bulls were housed at 18 to 20°C individually in metabolic cages with

Table 1. Ingredients and chemical composition of diet feed to German Holstein and Charolais bulls from 4 to 18 months of age

Component (% of air dry matter)	
Hay	25
Concentrate <sup>1</sup>	75
Chemical composition (% of dry matter)	
Organic matter	93.27
Crude protein	16.35
Crude fiber	13.62
Starch	18.91
Sugar <sup>2</sup>	10.42
Ether extract	2.72
N-free extract	60.58

<sup>1</sup>composition, dry matter basis: 44.8% barley, 36.9% sugar beet chips, 13.7% soybean meal (extracted), 3% molasses, 1.6% mineral and vitamin premix with 2.0% NaHCO<sub>3</sub>, 1.4% CaO, 0.25% NaH<sub>2</sub>PO<sub>4</sub>, 0.15% vitamin E mixture, 0.05% NaCl

<sup>2</sup>water soluble carbohydrates, calculated as monosaccharides

free access to water. The nutrition level was about 1.2 times maintenance. Daily feed rations were fed as equal meals, provided at 07.00 and 19.00 h. The diets were balanced out for the experimental period, including a preliminary period of 10 days and a sampling period of five days. Representative samples of roughage and concentrate were oven dried (65°C), ground to pass a 1-mm sieve, air equilibrated, and stored until analysis. Feed residues were collected daily, pooled over the sampling period, and prepared for analyses as indicated for the diet samples.

During the sampling period, all faeces were collected. At 24-h intervals, after weighing and thorough mixing, 10% (by weight) of the faeces was placed in a fridge at 4°C. The collections for each bull were composited. After homogenisation of the composited faecal samples, subsamples were taken to determine nitrogen (N) and dry matter (DM) content. Two further subsamples of about 750 g were lyophilised, pooled, ground through a 1-mm sieve, air equilibrated and stored for chemical analysis. DM of food,orts and faecal samples was estimated using a 65°C forced-air oven for 24 h and a 105°C oven for 3 h. The content of crude ash, crude fiber and crude fat of ground samples was estimated as described by Naumann and Bassler (1993). Crude fat determination included HCl-hydrolysis according to Kuhla et al. (1983). Content of N was estimated in dried feed and orts, fresh faeces and urine samples by Kjeldahl method (Naumann and Bassler, 1993).

**Period II (slaughtering).** The bulls were slaughtered at 18 months of age in experimental slaughterhouse of the institute according to a standardized procedure. The last feeding was carried out 3–4 h before. Intestinal tissue samples were obtained within 30 min after slaughter. Duodenal samples (about 10 cm in length) were taken from a site 50 cm distal of the pyloric sphincter, the jejunal ones from the beginning and mid-jejunum (approximate centre of the jejunum). Ileal samples were obtained 50 cm proximal of the ileo-caecal junction. Mucosal tissue was harvested by scraping intestinal tract samples with a glass slide. Samples of the rumen wall intended for morphological examination were obtained within 40 min after slaughter from the ventral ruminal sac (approximately 5 cm caudal of the *pila cranialis*). Furthermore, rumen liquor was collected. The rumen liquor were strained through four layers of gauze and used for determination of pH value, ammonia and short chain fatty acids (SCFA).

To estimate the nutrient and energy gain between four and 18 months of age, seven bulls of each

breed were slaughtered at an age of four months. Thus, the gain in the carcass was calculated as the difference between the protein and fat content at 18 and four months of age.

For SCFA analysis, a mixture of 5 ml rumen fluid and 2 ml iso-capronic acid (internal standard) was centrifuged at 3000 × g at 4°C for 20 min. The supernatant was then filtered (0.22 µm pore size) to measure the SCFA concentration by gas chromatography (GC-14A, Shimadzu, Kyoto, Japan) on an FFAP column (25 m × 0.25 mm i.d.). Ammonia was estimated by microdiffusion as described by Voigt and Steger (1967). The pH value was measured with a glass electrode (N 1042A, pH meter CG 841, Schott, Mainz, Germany).

For light microscopy and morphometry ruminal as well as intestinal samples (1 cm<sup>2</sup>) were fixed in 4% neutral formaldehyde solution. After rinsing with water, the samples were dehydrated in a graded series of absolute ethanol (30%, 50%, 70%, and 90%), cleared with benzene, saturated with and embedded in paraffin. Sections of 7 µm thickness (10 slices of each sample) were stained with haematoxylin per eosin. The length of 30 villi and depth of 30 crypts were determined by the computer operated *Image C* picture analysis system (Imtronic GmbH, Berlin, Germany) and the IMES analysis programme, using a colour video camera (SONY 3 CCD) and a light microscope (Axiolab, Carl Zeiss Jena, Germany). The same system was used to measure the length and width of rumen papillae and to estimate their number per cm<sup>2</sup> of mucosa. Total surface of papillae per cm<sup>2</sup> mucosa was determined as length × width × 2, multiplied by number of papillae per cm<sup>2</sup> (Hofmann and Schnorr, 1982).

## Statistical analyses

The results were analysed by descriptive statistics, *t*-test and regression analyses using the procedures of SPSS for Windows (Version 15.01, SPSS Inc., Chicago, USA). The results are presented as means ± SE.

## RESULTS AND DISCUSSION

### Animals and nutrient utilization

The bulls of both breed were fed *semi ad libitum* with an energy-rich diet (Table 1) to fully exploit their

genetically growth potential. There was no difference in the intake of DM and crude protein (CP) (Table 2). On the other side, BW, BW gain, and body protein gain were significantly greater in CH compared to H bulls. The CH bulls consumed less ( $P = 0.022$ ) DM per kg BW gain and less ( $P = 0.001$ ) protein per kg protein accretion than H bulls. No differences ( $P = 0.332$ ) was observed for body fat deposition. Details of fat and protein deposition in different organs and carcass tissues of both investigated cattle breeds were described by Pfuhl et al. (2007).

### Rumen fermentation and digestibility of nutrients

The different efficiency of feed utilization could be caused by differences in digestion processes. Therefore, the rumen fermentation and apparent digestibility of nutrients in total gastrointestinal tract were studied. The results are presented in Table 3 and Table 4. The pattern of SCFA, total concentration of SCFA, and pH-value in rumen juice were not significantly different between both breeds. The lower ( $P = 0.020$ ) ammonia concentration in rumen

Table 2. Feed intake, growth, and feed efficiency of German Holstein and Charolais bulls from 4 to 18 months of age (mean  $\pm$  SE;  $n = 6$ )

	German Holstein	Charolais	<i>P</i> -value
Intake			
DM (kg/day)	8.05 $\pm$ 0.23	8.17 $\pm$ 0.16	0.675
CP (g/day)	1294 $\pm$ 28.8	1307 $\pm$ 21.7	0.726
Growth			
Body weight (kg)	668.5 $\pm$ 21.6	763.6 $\pm$ 21.3	0.011
Body weight gain (g/day)	1223 $\pm$ 56.6	1385 $\pm$ 41.2	0.043
Body fat gain (g/day)	129.6 $\pm$ 15.6	153.5 $\pm$ 17.5	0.332
Body protein gain (g/day)	92.9 $\pm$ 4.1	128.3 $\pm$ 5.9	0.001
Energy accretion (MJ/day)	7.15 $\pm$ 0.62	8.80 $\pm$ 0.60	0.071
Feed efficiency			
DM intake/body weight gain (kg/kg)	6.62 $\pm$ 0.21	5.92 $\pm$ 0.15	0.022
CP intake/CP accretion (kg/kg)	13.81 $\pm$ 0.54	10.18 $\pm$ 0.48	0.001

DM = dry matter, CP = crude protein

Table 3. Short chain fatty acids, pH-value, and NH<sub>3</sub> concentration in rumen of German Holstein and Charolais bulls at 18 months of age (mean  $\pm$  SE;  $n = 6$ )

	German Holstein	Charolais	<i>P</i> -value
pH-value	6.7 $\pm$ 0.1	6.8 $\pm$ 0.1	0.777
SCFA (mmol/l)	95.6 $\pm$ 6.8	90.8 $\pm$ 7.0	0.652
Acetate (mol%)	69.8 $\pm$ 0.8	69.8 $\pm$ 0.6	0.991
Propionate (mol%)	16.4 $\pm$ 0.7	16.4 $\pm$ 0.8	0.979
Butyrate (mol%)	9.5 $\pm$ 0.8	9.3 $\pm$ 0.6	0.864
NH <sub>3</sub> (mmol/l)	11.3 $\pm$ 0.8	8.5 $\pm$ 0.9	0.020

SCFA = short chain fatty acids

Table 4. Digestibility of nutrients and content of digestible energy in German Holstein and Charolais bulls at 9 months of age (mean  $\pm$  SE;  $n = 6$ )

	German Holstein	Charolais	<i>P</i> -value
Organic matter (%)	76.1 $\pm$ 0.7	75.1 $\pm$ 0.9	0.383
CP (%)	65.5 $\pm$ 1.0	67.0 $\pm$ 0.6	0.227
EE (%)	61.7 $\pm$ 1.6	54.5 $\pm$ 2.6	0.041
CF (%)	61.0 $\pm$ 1.7	59.3 $\pm$ 1.8	0.511
NFE (%)	82.9 $\pm$ 0.5	81.8 $\pm$ 1.0	0.337
DE (MJ/kg Dry matter)	13.2 $\pm$ 0.13	13.1 $\pm$ 0.14	0.443

CP = crude protein, EE = ether extract, CF = crude fibre, NFE = N-free extracts, DE = digestible energy  
 DE (kJ) = 24.2  $\times$  digestible CP (g) + 34.1  $\times$  digestible EE (g) + 18.5  $\times$  CF (g) + 17.0  $\times$  NFE (g) (Jentsch et al., 2000)

fluid of CH bulls indicates slower breakdown of feed proteins and/or higher microbial protein synthesis. It could also be the result of a lower rate of endogenous urea transported into the rumen.

The apparent digestibilities of investigated nutrients were, with exception of ether extract, not significantly different between H and CH bulls (Table 4). Therefore, the content of digestible ener-

gy (DE) per kg consumed DM was the same for both breeds. From this results follows that the higher efficiency of DM intake utilization isn't the result of different digestion processes and intake of DE but must be justified by differences in post digestion processes or in the intermediary metabolism. Some hypotheses were discussed earlier (Kuhn et al., 2002; Bellmann et al., 2004).

Table 5. Number and size of rumen papillae and villus height and crypt depth of small intestinal mucosa in German Holstein and Charolais bulls at 18 months of age (mean  $\pm$  SE;  $n = 6$ )

	German Holstein	Charolais	<i>P</i> -value
Rumen papillae			
number ( $n/cm^2$ )	48.2 $\pm$ 2.02	46.3 $\pm$ 1.92	0.527
length (mm)	6.99 $\pm$ 0.21	7.13 $\pm$ 0.20	0.649
width (mm)	2.12 $\pm$ 0.07	2.26 $\pm$ 0.12	0.368
surface ( $mm^2/cm^2$ )	1 415 $\pm$ 31.7	1 477 $\pm$ 65.9	0.418
Duodenum			
villus ( $\mu m$ )	495 $\pm$ 16.5	586 $\pm$ 10.3	0.001
crypt ( $\mu m$ )	295 $\pm$ 8.2	358 $\pm$ 5.9	< 0.001
Proximal jejunum			
villus ( $\mu m$ )	518 $\pm$ 8.1	598 $\pm$ 8.4	< 0.001
crypt ( $\mu m$ )	292 $\pm$ 9.0	344 $\pm$ 16.7	0.020
Medial jejunum			
villus ( $\mu m$ )	534 $\pm$ 14.7	570 $\pm$ 9.6	0.071
crypt ( $\mu m$ )	304 $\pm$ 6.6	308 $\pm$ 6.6	0.642
Ileum			
villus ( $\mu m$ )	522 $\pm$ 10.9	471 $\pm$ 9.9	0.006
crypt ( $\mu m$ )	315 $\pm$ 6.3	322 $\pm$ 7.9	0.465

### Morphology of ruminal and intestinal mucosa and intestinal weight

The pivotal role of ruminal and intestinal tissues in both whole-body nutrient demand and specific tissue supply with metabolites like SCFA, amino acids, and glucose, is well-established (Reeds et al., 1999). It appears that splanchnic tissues like gastrointestinal tract largely compete with other body tissues for nutrients from the same arterial blood pool (Drackley et al., 2006). If this is so, the metabolism of nutrients in splanchnic tissues corresponds to metabolism of other tissues. We hypothesize, that gastrointestinal growth will be different in the two types of cattle breed. Furthermore, because the absorptive functions of the intestine are related to its morphology, alterations in intestinal morphology could be expected.

As seen in Table 5, there were no significant differences in all studied parameters of rumen mucosa between both cattle breeds. In contrast to ruminal

mucosa, the small intestine was different among the two breeds. The total weight of small intestine, calculated as percentage of BW, was lower ( $P = 0.002$ ) and the CP content of the proximal jejunum was higher ( $P = 0.032$ ) in CH compared to H (Table 6). In CH the villi were higher in duodenum ( $P = 0.001$ ) and proximal jejunum ( $P < 0.001$ ), the crypts were deeper in the same intestinal mucosa ( $P < 0.001$  and  $P = 0.020$ ) compared to H. The villi of CH bulls were lower in ileum ( $P = 0.006$ ).

As can be seen in Figure 1, positive correlations exist between CP-utilization and morphological parameters of small intestinal mucosa. The observed morphological differences of small intestine between both types of cattle suggest that the duodenum and proximal jejunum of CH bulls adapt to increase the absorptive surface due to the increase in nutrient demand associated with protein deposition. Moreover, the small intestine of CH bulls appears to use less energy and protein in their contribution to whole body metabolism in com-

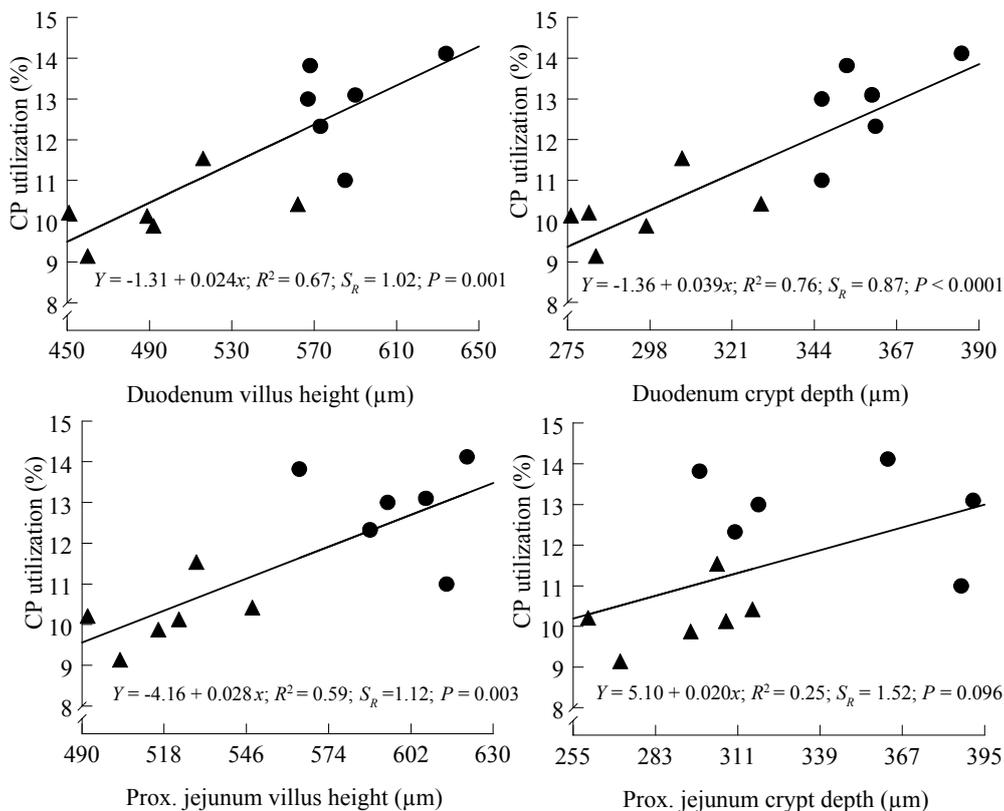


Figure 1. Relation between crude protein (CP) utilization (CP accretion/CP intake) and small intestinal villus height and crypt depth, respectively

▲ German Holstein, ● Charolais

Table 6. Crude protein concentration of small intestinal mucosa and weight of small intestine of German Holstein and Charolais bulls at 18 months of age (mean  $\pm$  SE;  $n = 6$ )

	German Holstein	Charolais	P-value
Crude protein (% of wet weight)			
Duodenum	12.28 $\pm$ 0.54	12.25 $\pm$ 0.26	0.961
Proximal jejunum	11.89 $\pm$ 0.27	13.20 $\pm$ 0.45	0.032
Medial jejunum	12.16 $\pm$ 0.44	12.41 $\pm$ 0.28	0.652
Ileum	11.93 $\pm$ 0.38	12.04 $\pm$ 0.46	0.862
Weight of total small intestine (% of body weight)	1.08 $\pm$ 0.07	0.79 $\pm$ 0.02	0.002

parison to H bulls, because the weight of total small intestine (Table 6) and gut fat depot (Pfuhl et al., 2007) are significantly lower. Figure 2 shows the negative correlation between intestinal size and CP utilization ( $P = 0.004$ ). Cant et al. (1996) reviewed that the energy demands of intestine for protein turnover and ion transport depend on intestinal size. The lower weight of total small intestine appears to be a response to the changed epithelial surface in duodenum and proximal jejunum. The results explain the lower energy requirement for maintenance of CH bulls compared to H bulls as demonstrated by Chudy (2001).

## CONCLUSION

The higher growth potential and the higher feed efficiency in CH bulls compared to H bulls are not accompanied by differences in apparent digestibility of nutrients in total tract and pattern of ruminal

volatile fatty acids, but in weight of small intestine, and morphology of small intestinal mucosa. It seems, that the small intestine adapt to meet the nutrient needs of the animal. The small intestine appears to use less energy and protein in their contribution to whole body metabolism if growth potential increases. Additional research is needed both in the area of protein turnover and energy use of intestine in dependence of nutrient requirement of the animal.

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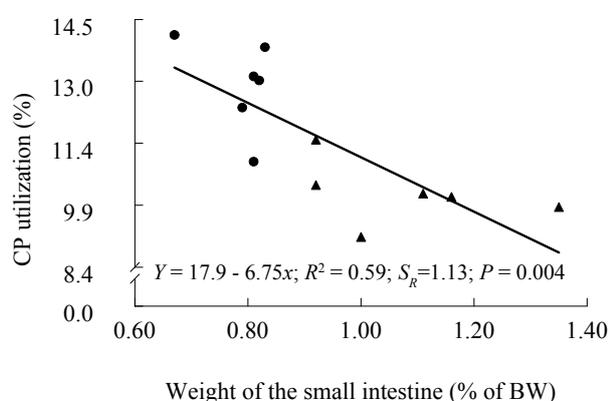


Figure 2. Relation between crude protein (CP) utilization and weight of small intestine

▲ German Holstein, ● Charolais

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