Effects of partly defatted insect meal (*Hermetia illucens*) or micro algae (*Spirulina platensis*) in mixed diets on intestinal mucosal surface and mucin secretion of meat type chicken

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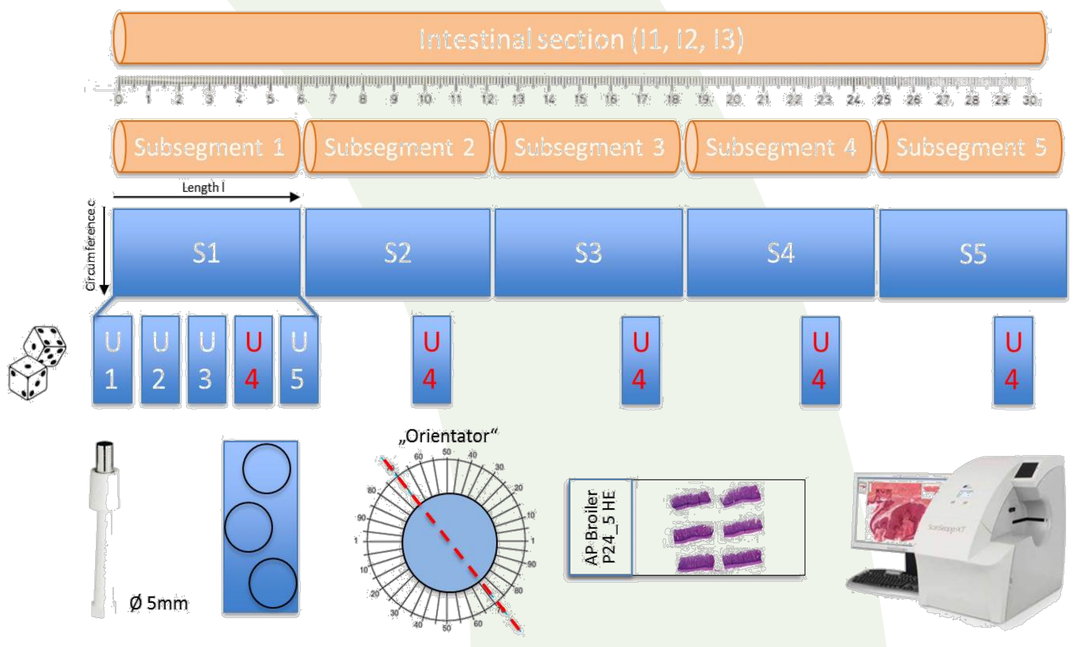
Aim of the study

Insects or algae are promising alternatives to replace soybean meal (SBM) in animal nutrition. As part of the multidisciplinary project “sustainability transitions” the study aimed to investigate effects of replacing 50% SBM by partly defatted Hermetia meal (HM) from larvae of the black soldier fly *(Hermetia illucens)* or blue green algae *(Spirulina platensis)* meal (SM) in mixed chicken diets on mucosal surface and microstructure by unbiased stereological morphometric analysis of the small intestine.

Material and Methods

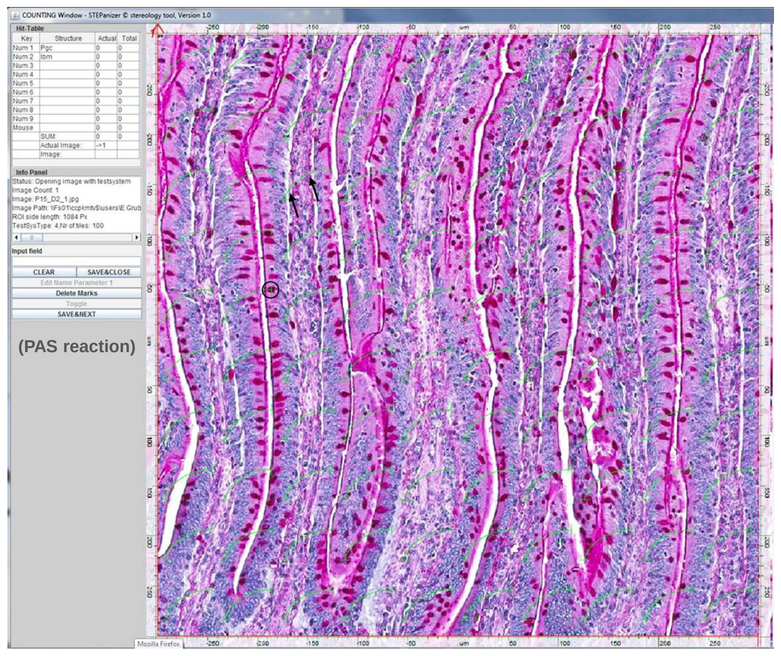
Animals: 180 one-day-old male growing chickens (Ross 308); 6 birds per pen
Growth study (34d) : 3 diets; feed/water supply on free choice level; 8 birds per diet slaughtered after 12 hrs fastening (n=24)
Control diet: starter (1-21d)/ grower (22-34d) diets with 39/32% SBM; basic supplementation of Lys and Met
HM & SM diet: 50% of SBM replaced by HM or SM, respectively, with basic AA fortification (Lys, Met) according to control diet[1]
Preparation of the small intestine: I1: duodenum, I2: proximal jejunum (up to Meckel’s diverticulum), I3: distal jejunum/ileum



Systematic uniform random sampling (SURS)[2]: for stereological analysis of the intestinal sections and calculation of the primary mucosal surface area S_{pm}

S_{pm} = length l x mean circumference c

Histology: 5 SURS sub-segments (U) per section (S) were formalin-fixed (4%) and from each, 3 biopsies (Ø 5mm) were punched out/ divided at random position/ orientation, paraffin-embedded for serial vertical sections (4 µm) and stained with hematoxylin eosin (HE) or Periodic acid-Schiff (PAS) reaction



Stereological morphometry: analysis of SURS-generated vertical visual fields from digitalized slides with a stereological software tool (STEPanizer©); counting of different Intersections (I) and Pointcounts (P) for determination of the villus amplification factor ($Ss_{(v,pm)}$) and estimation of the villus surface area **$S_v = S_{pm} \times Ss_{(v,pm)}$**
as well as for calculation of epithelial mucin volume $Vv_{(muc)}$ to basement membrane surface $Ss_{(bm)}$ ratio by **$Vv/Ss = \frac{P(mucin) \times l(p)}{2 \times l(BM)}$**

Statistical analysis: one-way ANOVA with Kruskal-Wallis multiple comparisons test (GraphPad Prism V5).

Results

The final body mass (BM) differed significantly ($p \leq 0.01$) between treatments (control: 2439.7^{c} ±317g; HM: 1597.5^{b} ±105g; SM: 1195.2^{a} ±186g). Compared to control diet, the relative S_{pm} was significantly enlarged in all intestinal sections with diet SM, and in I1 with diet HM. However, due to balancing effects by the villus amplification factor ($Ss_{(v,pm)}$) significant differences of relative S_v data were only observed in I1 between control and SM diet.
Mucin volume to surface ratios tended to be lower in all intestinal sections with diet HM.

	Control (n=8)			HM (n=8)			SM (n=8)		
	I1	I2	I3	I1	I2	I3	I1	I2	I3
relative S_{pm} (cm^2/g BM)	0.03^{a} (12%)	0.06^{a} (6%)	0.04^{a}(14%)	0.04^{b} (16%)	$0.07^{a,b}$(13%)	$0.05^{a,b}$ (8%)	0.05^{b*}(16%)	$0.09^{b\dagger}$(13%)	0.06^{b*}(8%)
$Ss_{(v, pm)}$	19.1 (38%)	36.8 (30%)	23.1 (27%)	19.1 (19%)	29.1 (38%)	18.5 (46%)	17.7 (21%)	27.3 (27%)	20.2 (34%)
relative S_v (cm^2/g BM)	0.48^{a} (49%)	2.10 (31%)	0.95 (35%)	$0.69^{a,b}$(31%)	2.06 (39%)	0.96 (46%)	0.80^{b} (20%)	2.50 (30%)	1.22 (35%)
$Vv_{(muc)}/Ss_{(bm)}$ (cm^3/cm^2)	3.75 (33%)	4.36 (32%)	7.44 (22%)	2.61 (37%)	3.85 (39%)	6.98 (31%)	3.33 (24%)	4.48 (43%)	8.32 (21%)

Mean values and coefficients of variation (CV) in %; means in the same row of the corresponding column (I1, I2, I3) with different superscript letters are significantly different ($p \leq 0.05$; $^{\dagger}p \leq 0.005$; $^{*}p \leq 0.001$).

Conclusion

Results indicate that 50% algae meal based diets induce an increase in intestinal absorption surface, especially in the duodenum, possibly due to rather inefficient protein digestibility. The observed trend to lower mucosal mucin volume with insect meal based diets might point to improved intestinal health. Further modifications of intestinal microstructure are under investigation in ongoing experiments.

[1]Velten et al.: Proc. Soc. Nutr. Physiol. 26, 2017, 89; [2]Makanya et al: J. Anat. 187, 1995, 361-368

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