Role of a Carbohydrate Binding Module from *Clostridium thermocellum Ct*Lic26A in the function of a recombinant cellulase used to supplement a barley-based diet for broiler chicks

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INTRODUCTION

Cellulases and xylanases display a modular architecture that comprises a catalytic module linked to one or more non-catalytic Carbohydrate-Binding Modules (CBMs). CBMs have been classified, based on primary structure similarity, into 52 different families. These non-catalytic modules mediate a prolonged and intimate contact of the enzyme with the target substrate eliciting efficient hydrolysis of the target polysaccharides.

A study was undertaken to investigate the importance of a family 11 CBM, displaying high affinities for barley β-glucans, in the function of recombinant derivatives of cellulase *Ct*Lic26A-Cel5E of *Clostridium thermocellum* (Figure 1) used to supplement a barley-based diet for broiler chicken. Truncated forms of the modular cellulase *Ct*Lic26A-Cel5E, with or without the enzyme's β-glucan-binding domain, were produced and used to supplement a barley-based diet for broiler chicks. The basal diets were prepared, supplemented with the required enzymes and used to feed broiler chicks *ad libitum* from days 1-28.

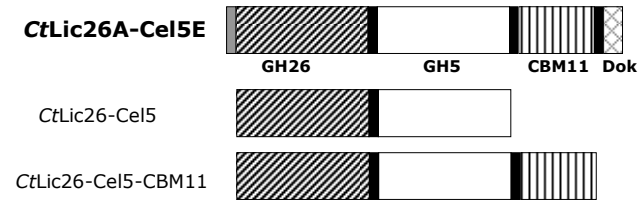

Figure 1. Domain organization of *Ct*Lic26A-Cel5A and its truncated derivatives Lic26-Cel5 and Lic26-Cel5-CBM11 used in this study. The β-glucanase (GH26), cellulase (GH5), β-glucan binding domain (CBM11) and the dockerin (Dok) are indicated. The gray and the black boxes represent the linker sequences and the signal peptide, respectively.

RESULTS

Data show that birds fed on diets containing the recombinant *Ct*Lic26A-Cel5E modular derivatives or the commercial enzyme mixture RovabioTM Excel AP display improved performance when compared with birds fed on diets not supplemented to exogenous enzymes ($P<0.05$) (Table 1).

Enzyme supplementation had no effect on crop, gizzard and liver relative weights or on the duodenum, jejunum and caecum lengths. In contrast, ileum relative length was significantly reduced ($P<0.05$) in birds receiving the commercial enzyme mixture. In addition, diet supplementation with exogenous β-glucanase activities significantly contributed to decrease the viscosity of small intestine contents.

Cellulase activity could be detected along the entire digestive tract of most animals fed on diets supplemented with the plant cell wall hydrolases (Figures 2 and 3).

Table 1. Growth performance of broilers fed on a barley-based diet not supplemented (C0) or supplemented with a commercial cellulase mixture (Rov) or truncated derivatives of *C. thermocellum Ct*Lic26A-Cel5E β-glucanase containing (Lic26-Cel5-CBM11) or not containing (Lic26-Cel5) a family 11 CBM.

	C0	Rov	Lic26-Cel5E	Lic26-Cel5E-CBM11	SEM	p(*F*)
Body Weight (g)						
0d	42.4	42.6	42.2	42.1	0.152	NS
7d	142.8^{b}	154.9^{a}	154.1^{a}	148.1^{ab}	3.209	0.028
14d	331.7^{b}	366.7^{a}	362.6^{a}	355.5^{a}	8.106	0.014
21d	674.1^{b}	739.7^{a}	725.1^{a}	732.6^{a}	13.426	0.004
28d	1158.0^{b}	1243.4^{a}	1240.4^{a}	1247.9^{a}	21.313	0.009

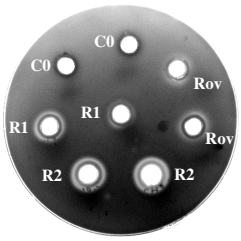

Figure 2. Detection of β-glucanase activity in the crop contents of broilers fed on barley based diet not supplemented (C0) or supplemented with a commercial cellulase mixture (Rov) or truncated derivatives of *C. thermocellum Ct*Lic26A-Cel5E β-glucanase containing (R2, enzyme Lic26-Cel5-CBM11) or not containing (R1, enzyme Lic26-Cel5) a family 11 CBM.

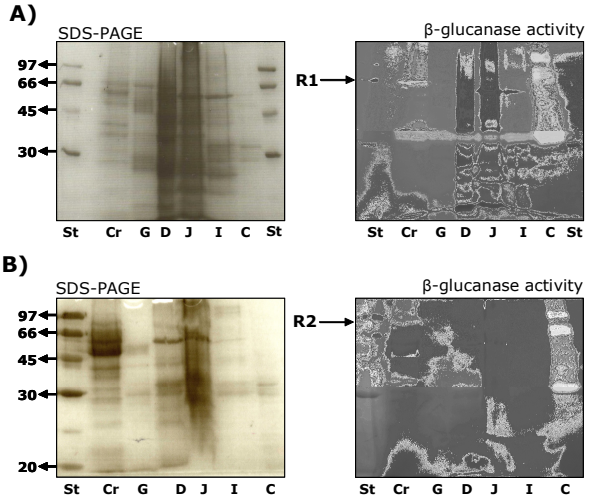

Figure 3. Zymogram analysis of digesta samples collected from various regions of the GI tract of birds fed on a barley-based diet supplemented with the recombinant β-glucanases Lic26-Cel5 (A) or Lic26-Cel5-CBM11 (B). Proteins were fractionated through SDS-PAGE and stained for β-glucanase activity after enzyme renaturation. Abbreviations: St, low molecular weight protein standards; Cr, crop; G, Gizzard; D, duodenum; J, Jejunum; I, Ileum; C, Caecum. The location of the two exogenous recombinant enzymes is highlighted (R1, Lic26-Cel5 and R2, Lic26-Cel5-CBM11).

CONCLUSIONS

1. **Individual recombinant cellulases could be as effective as complex mixtures of glycoside hydrolases in attenuating the depreciative effects of soluble polysaccharides found in barley-based diets.**
2. **When incorporated at high dosage rates (30 U/kg of basal diet) a tri-modular cellulase containing a family 11 CBM and its double-domain counterpart consisting on the enzyme's catalytic modules have equal capacities to improve the nutritive value of a barley-based diet.**
3. **Both recombinant enzymes were prone to peptidolysis in the birds' GI tract. It is suggested that this process, although not affecting the capacity of the resulting enzymes to degrade the anti-nutritive β-glucans *in vitro*, could have influenced the capacity of *Ct*Lic26-Cel5-CBM11 to act *in vivo*.**
4. **The capacity of the non-catalytic family 11 CBM to elicit the function of *Ct*Lic26A-Cel5E recombinant derivatives, when incorporated at lower dosages rates in barley-based diets for poultry, is currently under investigation.**

REFERENCE

Guerreiro *et al.* (2008) *British Poultry Science, in press*

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