Effect of Natural Carbohydrate Fraction from Yeast Cell Wall (NCF) and Age on Intestinal Morphometric Parameters of Broiler Chickens

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Abstract

Yeast cell wall products are used as feed supplements for increasing animal health and performance. A trial was set up to test effects of natural carbohydrate fraction from yeast cell wall (NCF) on gut parameters that can be correlated with gut health. Trial design included four treatments (control group and three groups with different levels of NCF in feed: 0.02%, 0.04% and 0.08%) and eight replications per treatment. Each replication was one pen with 38 broiler chicks. Chicks were sexed in incubation station and only males were used in the trial. On days 7 and 28 of the trial, after body weight had been measured, chicks with weight approximate to that of their treatment average were selected for intestinal sampling. Ten chicks per treatment were slaughtered. Central part of jejunum was fixed, sectioned and stained with double staining (Alcian Blue and Periodic Acid-Schiff). At least 15 measurements (25 on average) were made per bird for villus height and width, crypt depth and *Tunica muscularis* thickness. Statistical analysis was performed using factorial ANOVA test with feed and age as factors, and with Duncans' test. Age significantly affected jejunal morphometry, while effects of NCF were less obvious. Villus height, crypt depth and *Tunica muscularis* thickness doubled from 7th to 28th day, leaving villus to crypt ratio unchanged. Villus width also increased, but to a lesser extent, making villi of older chicks more elongated. Group receiving 0.02% NCF in feed had smaller crypt depth, and larger villus to crypt ratio, while group receiving 0.08% NCF had smaller villus area (villus height × villus width) compared to the control group.

Keywords: broilers, intestinal morphology, mannan-oligosaccharides

1. Introduction

Mannan-oligosaccharides (MOS) from yeast cell wall (YCW) of *Sacharomyces cerevisiae* are often used as feed ingredients with purpose to improve animal health and performance. Their effects on performance of different species of domestic monogastric animals have been tested in huge number of trials. Around 170 comparisons have been summarized in three large meta-analyses [1-3].

It is hypothesized that one of the modes of action of YCW products is trough improving intestinal health, and that those effects could be observed trough measurements of intestinal morphology. Naturally occurring intestinal bacteria stimulate the development of villus microvasculature [4], which could be the reason why normally raised chicken have larger villus length and villus area compared to chickens that are raised under conditions of reduced bacterial load [5]. It was hypothesized that higher villus height could be the result of increased population of beneficial bacteria [6], and that it could be used as a sign of good intestinal health.

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In many researches, addition of yeast cell wall products in feed have not induced statistically significant changes in intestinal morphology [7-9], while in others it have led to longer villi and larger villus to crypt ratio [10-14]. Only on rare occasions YCW products resulted in less wide jejunal villi of broiler chicks [15] and shorter duodenal villi of pigeons [16]. Sometimes, statistically significant difference have been observed between groups receiving different levels of YCW products, while differences between experimental groups and control group were not significant, as in research done on rabbits [17].

New generation of YCW product (Actigen™, Alltech Inc.) is described as a natural carbohydrate fraction produced from the cell wall of a specific strain of *Sacharomyces cerevisiae* (NCF). Brümmer *et al.* [18] have shown that NCF can induce what is considered to be a positive intestinal change, at quite low inclusion levels of only 0.1 to 0.2 g of NCF per kg of feed. Recent research showed a greater improvement in broiler performance with inclusion level of 0.4 compared to 0.2 g of NCF per kg of feed [19].

The objective of this study was to determine the effect of three inclusion levels (0.2, 0.4 and 0.8 g/kg) of NCF and two different bird ages on parameters that could be linked with intestinal health

2. Materials and methods

Broiler chickens used for morphometric study have been taken from larger production trial. Trial design included four treatments (control group and three groups with different levels of NCF in feed: 0.02%, 0.04% and 0.08%) and eight replications per treatment. Each replication was a straw bedded pen with 38 Ross 308 broiler chicks. Chicks were sexed in incubation station and only male chicks were used in the trial. Corn-soybean based diets, balanced according to commercial recommendation were offered ad libitum.

On days 7 and 28 of the trial, after body weight had been measured, broiler chicks with individual body weights approximate to that of their treatment average were selected for the intestinal sampling. Ten chicks per treatment were slaughtered. Central part of jejunum was fixed, sectioned and stained with double staining (Alcian Blue and Periodic Acid-Schiff) (Figure 1). Histological parameters were determined using light microscope and software for image analysis (IM1000 Image Manager, Leica). At least 15 measurements (25 on average) were made per bird for villus height and width, crypt depth and *Tunica muscularis* thickness. Villus width was measured at three points: close to the bottom, at the midpoint and close to the tip of the villus and the average of these three measurements was used in the statistical analysis. Villus area was calculated by multiplying villus height with average villus width.

All procedures were conducted following the ethical norms proposed by European convention for the protection of vertebrate animals used for experimental and other scientific purposes, confirmed by Serbian authorities [20].

Statistical analysis was performed using factorial ANOVA test with feed and age as factors, and with Duncans' test in Statistica software (StatSoft Inc., version 8.0, 2008).

3. Results and discussion

Measured intestinal parameters, together with results of statistical analysis are shown in Table 1. Villus height, crypt depth and *Tunica muscularis* thickness approximately doubled from 7th to 28th day, while villus to crypt ratio remained unchanged. Villus width also increased, but to a lesser extent, making villi of older chicks more elongated. All values were within usual range for broiler chicken of given age [9, 12, 13, 15], confirming that intestinal health of chicks in this study was at usual level, broilers neither being overprotected nor overexposed to presence of common bacteria.

Group receiving 0.02% NCF in feed had smaller crypt depth, and larger villus to crypt ratio (Figure 1), that is usually considered beneficial. Difference was smaller at 7 days of age, and increased at 28 days of age (data not shown). It was suggested that increased crypt depth could indicate higher enterocyte migration to replace damaged ones [12], or higher goblet cells production in crypts [21]. It was also observed that birds exposed to more bacteria had deeper crypts [5]. However, in previous trial [18] crypt depth was not affected by NCF supplementation.

While group receiving 0.02% of NCF in feed had that could be interpreted as positive changes compared to control group, group receiving 0.08% NCF had smaller villus area (villus height × villus width) compared to the control group, similarly as in some previous studies [15, 16]. Significant interactions between NCF addition and bird age on intestinal parameters were not found.

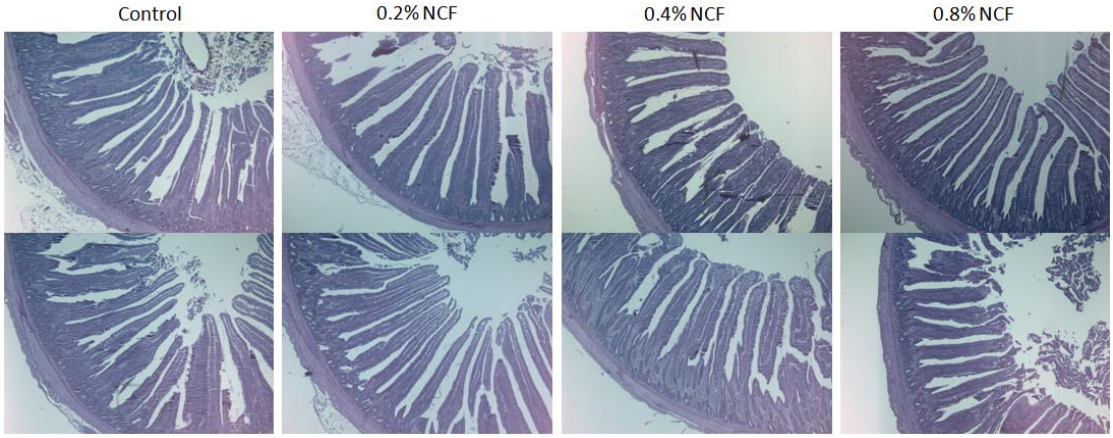

Figure 1. Examples of cross sections of jejunum of 28 days old chicks consuming different levels of NCF in feed

Table 1. Effect of age and NCF supplementation on jejunal morphometric parameters of broiler chickens

	Villus height (μm)	Villus width (μm)	Villus area (mm^2)	Crypt depth (μm)	Villus/cryph ratio	*T. musc*. thickness (μm)
			Age			
Day 7	518^{b}	97^{b}	0.050^{b}	138^{b}	3.81	90^{b}
Day 28	1139^{a}	149^{a}	0.169^{a}	280^{a}	4.32	179^{a}
			NCF addition			
Control	862	132	0.123^{a}	232^{a}	3.90^{b}	140
0.2% NCF	899	118	0.114^{ab}	194^{b}	4.69^{a}	128
0.4% NCF	792	128	0.110^{ab}	206^{ab}	3.89^{b}	132
0.8% NCF	787	119	0.098^{b}	208^{ab}	3.85^{b}	139
S.E.M.	40.6	4.05	0.008	9.84	0.137	6.13
			P values			
Age	0.000	0.000	0.000	0.000	0.065	0.000
NCF	0.328	0.264	0.210	0.110	0.122	0.585
Age x NCF	0.634	0.264	0.392	0.241	0.342	0.820

a.b Means within a continuous column with different superscript are significantly different (P<0.05)

4. Conclusions

Age significantly affected jejunal morphometry, while effects of NCF were subtler. NCF effects were also variable between groups receiving different levels of NCF. It could be assumed that YCW products like NCF have complex modes of action trough the interaction with both host animal and intestinal bacteria, and are not easy to quantify.

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