Summary

A study was conducted to evaluate a natural carbohydrate fraction (NCF) derived from mannan oligosaccharide in feed on growth performance, intestinal morphology and goblet cell number and area of male broilers. Dietary treatments included: 1) control diet (antibiotic and NCF free), 2) NCF at 200g/t, 3) NCF at 400g/t, and 4) NCF 800g/t. Birds were placed into 12 replicate pens/treatment (5 birds/pen). Body weight and feed intake were recorded weekly to day 42. At this time a 2.5cm section of jejunum and duodenum were excised post mortem for morphological analysis. Birds fed 200g/t and 800g/t NCF were significantly (P<0.01) heavier from day 14 onwards than the control birds. Feed intake was significantly increased for birds fed 200g/t NCF over the control in weeks 3 and 5 (P<0.05). Diet including 200g/t and 800g/t of NCF significantly decreased the FCR over the control in the first phase (1-14 days) (P<0.01), in the second phase all inclusion levels of NCF decreased FCR (P<0.05). NCF had no significant effect on villus height, villus width, crypt depth or villus to crypt ratio in either duodenum or jejunum. NCF did not significantly affect goblet cell area or goblet cell number in the duodenum, however in the jejunum 800g/t NCF significantly (P<0.05) increased goblet cell area over the control. In conclusion under the conditions of this trial NCF showed a positive effect on performance in the starter and grower phases, and increased goblet cell area in the jejunum, suggesting higher levels of mucin production. This indicated that the performance benefit of NCF could be age-dependent with younger birds responding more than the older ones. In the conditions of the poultry research unit, there were no additional benefits to performance when feeding NCF for a longer period (post 4 weeks), however it is postulated that birds fed NCF would have greater defence to pathogenic challenge through increased storage capacity of mucin.

Key words: Yeast, feed additive, prebiotic, crypt depth, villi height, performance, goblet cell
INTRODUCTION

Over the past few years there has been increased concern about antibiotic resistant bacteria and the inclusion of antibiotics in animal diets for growth promotion. This led to the ban of a number of antibiotics growth promoter (Dibner & Richards, 2005). Since the EU ban on using antibiotic as growth promoters in 2006 (Huff, et al., 2006), there has been an increase in the incidence of endemic diseases in poultry (Chee, 2008), in addition to reports of slower growth and higher disease challenges causing significant economic losses (Thomke & Elwinger, 1998) and negative welfare implications for poultry. In particular the gut health has been affected. Without a healthy intestinal tract a broiler cannot reach its full performance potential. Due to this there has been a drive in the market for feed supplements that will improve health and production of poultry but remain safe for humans. The morphological structure of the gastrointestinal tract (GIT) offers key information to judge gut health. Longer, thinner villi are considered to indicate that the bird will have a better ability to absorb nutrients, due to the increased surface area (Gao, et al., 2008). Shorter villi height (VH) and deeper crypt depth (CD) are associated with decreased digestibility of nutrients (Zhang, et al., 2005). Deeper CD are considered a negative indication of gut health because new epithelial cells are produced in the crypts and migrate along the villi to the tip (Gao, et al., 2008), therefore deeper CD indicates that there is a higher tissue turnover of epithelium cells. It is thought that the faster turnover of tissue is due to the host’s compensating for villus atrophy due to inflammation resulting from pathogens and their toxins (Gao, et al., 2008).

Mannan oligosaccharides (MOS) are mannose-based carbohydrates it can be derived from yeast cell walls and have a prebiotic function (Chee, 2008). MOS has been shown to have a positive effect on gut heath, by binding to enteropathogens, inhibiting their proliferation and stimulating specific microbial populations in the GIT (Spring, et al., 2000; Kocher, et al., 2004). This leads to
increased VH and decreased CD, which may in turn improve nutrient absorption (Santin et al. 2001; Sims, 2004; Mourão et al., 2006). Goblet cells are found in the epithelial layer along the villi of the bird’s GIT. Goblet cells produce and secrete mucin glycoproteins that make up part of the mucus layer, which protects the intestinal surface against damage by bacterial and environmental toxins, microorganisms and some coarse dietary components (Santos et al., 2007). Several studies have also found that feeding MOS increases the number and/or the volume of goblet cells found in the small intestinal tract (Baurhoo, et al., 2009; Brummer, et al., 2010). It has not only been found that MOS has increased the number of goblet cells but also their size (Uni and Smirnov, 2006). Mucin is also thought to be beneficial to developing the innate immune system (Koutsos and Arias, 2006).

Actigen™ (Alltech Inc. Nicholasville, Kentucky, USA), is a specific natural carbohydrate fraction (NCF) that has been derived from the cell wall of saccharomyces cerevisiae. NCF should contain a high affinity for the mannose-specific type-1 fimbriae of pathogenic bacteria such as Escherichia coli. (Ofek, et al., 1977) and salmonellae (Spring, et al., 2000; Miguel, et al., 2004). The objective of this study was to evaluate the effect of this specific natural carbohydrate fraction isolated from yeast cell wall oligosaccharides, on VH, CD and the goblet cell profiles of broilers.

**MATERIALS AND METHODS**

Two hundred and forty day-old male Ross 308 birds were placed in 48 different pens, each containing 5 birds and fed 1 of 4 dietary treatments (12 replicates for each treatment). The diets were randomly allocated to remove any effect of the room environment on the study. The room temperature was initially adjusted to 32°C and then gradually lowered to reach approximately 21°C by d 21. Temperature was monitored on a daily basis and light was 23D:1L for the duration of the experiment. Birds were kept with a stocking density aiming for a 30kg per m² on day 42. Birds
were provided with *ad libitum* access to water and feed. Feed was a wheat/soya based mash containing no enzymes or coccidiostats. The trial lasted 42 days with a 3 phase feeding programme, starter (1 to 14 d); grower (15 to 28 d) and finisher (29 to 42 d). The basal diet was formulated and made by Target feeds (Coton, Whitchurch, Shropshire, UK), formulations are shown in table 1.

The dietary treatments were then added to the basal diet. The 4 dietary treatments were; 1) control diet (antibiotic and NCF free), 2) NCF at 200g per tonne, 3) NCF at 400g per tonne, and 4) NCF 800g per tonne. Weekly and overall feed intake and weight gain was recorded of birds prior to culling on day 42. At this time a 2.5cm section of jejunum and duodenum were removed and immediately rinsed with PBS solution. The tissue was then placed in Bouin’s fixative for 8 hours and then stored in 70% industrial methylated spirits. The tissue samples were then embedded in paraffin and cut at 8µm using a rotary microtome (Leitz Wetzlar 1512 microtome Leitz, Milton Keynes, Bucks, UK). The sections were stained with a combination of 1% alcian blue (pH 2.5) and periodic acid-Schiff’s reagent. The following measurements were taken using a light microscope; CD, villus height (VH), villus width (VW), villus/crypt ratio (VCR), and goblet cell area and number of the jejunum. VH was measured as the length between the villus-crypt axis and the tip of the villus (20 villi per sample, 240 per treatment). VW was measured at the midpoint between the villus-crypt axis and the tip of the villus. CD was measured from the villus-crypt axis to the base of the specific crypt. Goblet cell area was measured as the “cup” area of the goblet cells (µm²). Two hundred measurements (10 measurements on 20 villi) were made for each intestinal sample. Goblet cell density was determined as the number of goblet cells per 165µm. Gut morphology was analysis using an Olympus BX51 microscope fitted with an Olympus DP71 camera (Olympus Microscopy, Essex, UK) and Cell F software (Olympus Europa GmbH, Hamburg) was used for all measurements. Gut
morphology measurements and performance data were analysed using SPSS software version 12 for Windows; First the data was analysed for normality. If the data was normally distributed a 1-way ANOVA was used, if the data was not normally distributed a Kruskal-Wallis test was performed. Treatment means were separated using the Bonferroni’s post hoc test, and statistical significance was declared at $P < 0.05$. Institutional and national guidelines for the care and use of animals were followed and all experimental procedures involving animals were approved by the Nottingham Trent University College of Science ethical review committee.

RESULTS AND DISCUSSION

The live body weight is presented in table 2. Significant (P<0.01) differences between treatments were observed for body weight on days 14, 21, 28, 35 and 42, generally 200g/t and 800g/t NCF increased live weight over the control. Feed intake was significantly increased for birds fed 200g/t of NCF over the control in weeks 3 and 5 (respectively P = 0.021, P = 0.025, data not shown). The FCR is presented in table 2, a significant difference was observed for the first and second phase of feeding. Diet including 200g/t and 800g/t of NCF significantly decreased the FCR over the control in the first phase (P<0.01), whereas in the second phase all inclusion levels of NCF decreased FCR (P<0.05). However the FCR seen in week one for the control and 400g/t NCF is abnormally high for nutritionally complete diets, indicating excessive spillage.

Morphometric measurements from the stained slides are shown in table 3. It was observed that NCF had no significant effect on VH, VW, CD or VCR in either the jejunum or duodenum.

When looking at the goblet cell number and area in the duodenum (table 4), it is observed that the diets including NCF did not significantly affect goblet cell area, goblet cell number per 165μm or goblet cell measurements as a ratio, however in the jejunum 800g/t NCF significantly (P<0.05) increased goblet cell area over the control.
Supplementation of 200g/t and 800g/t of NCF significantly increased weekly bird body weights compared to the control diet from 14 days, but the inclusion level of 400g/t NCF did not significantly change body weight from the control. This indicates that there may be two mechanisms behind the observed response to supplementation; one occurring at the lowest inclusion and one occurring at the highest inclusion, with apparently antagonist effects at the middle inclusion level. This contradicts both Reisinger et al. (2012) and Gao et al. (2008) who found a positive quadratic response in broiler body weight to a yeast derivative and yeast cell culture respectively. The authors suggest an explanation for these findings is that higher inclusion levels may be interacting with the immune system causing energy to be partitioned towards the immune system, rather than supporting growth. Whilst this mechanism may explain the observed response at 400g/t NCF, it does not support the improved performance at 800g/t NCF.

Increased body weight following supplementation with MOS (such as that observed at 200g and 800g/t in the current study) is often attributed to reduced effects of pathogenic bacteria in the intestinal tract as the binding of pathogenic bacteria to MOS results in their evacuation from the intestine with other non-digested feedstuffs (Spring et al., 2000). This may have reduced sloughing of villi in birds fed MOS thereby contributing to increased performance compared to the control, due to an increased capacity to absorb nutrients (Sun et al. 2005). When feeding NCF to broilers it could be postulated that birds will also have longer VH and shorter CD via a similar reduction in sloughing, because Spring et al. (2000) reported that pathogens with the mannose-specific type-1 fimbriae, such as some strains of *Escherichia coli*, *Salmonella typhimurium* and *Salmonella enteritidis*, are attracted to mannans, which are reported to be present in NCF (Che, et al., 2012), and readily bound with them instead of attaching to intestinal epithelial cells (Castillo, et al., 2008). Therefore these pathogenic bacteria cannot colonise the GIT and release toxins. These bacterium
and their toxins can cause inflammation that in turn cause atrophy of the epithelial cells of the villi (Gao, et al., 2008), thus reducing the absorptive function of the gut through shorter VH and deeper CD (Yason, et al., 1987). If the NCF bind the pathogenic bacteria and reduce the level of these bacteria in the GIT, there will be less villi damage in the gut therefore improving the gut health of the bird. To compensate for this atrophy the bird has to increase its tissue turnover, and as epithelial cells are produced in the crypts and migrate along the villi to the tip, it is thought that the higher turnover in the crypt cell cause it to become deeper (Gao, et al., 2008). Therefore shallower CD are considered a good indicator of gut health. MOS are also thought to increase the number of beneficial bacteria in the gut.

Morphometric analysis at 6 weeks revealed that NCF had no significant effect on the gut morphology of the birds at this time point. A possible reason why no effect was evident in the gut morphology and performance of birds fed NCF at 6 weeks, may be due to the birds not being challenged with pathogenic microbes at this point. This trial supports the VH findings of White, et al. (2002) in pigs and Yitbarek et al. (2012) and Sohail et al. (2012) in broilers when feeding MOS. However, Iji et al. (2001) and Zhang et al. (2005) found that birds fed yeast cell wall fractions had longer VH than control birds at 21 days. Similarly, Baurhoo et al. (2007) measured VH and found that MOS improved VH at 28 days but not at 42 days. A similar, early age response may have occurred in this present study, but as histological measures were not taken at 21 or 28 days this cannot be verified.

When looking at CD, Zhang et al. (2005), Yitbarek et al. (2012) and Sohail et al. (2012) found feeding MOS had no effect on CD in birds, which was also shown in this study. Santin et al. (2001) also saw no effect of MOS at 42 days, however, decreased CD was seen when feeding MOS at 7 days. In addition, this present study found no effect on VCR, which can be used as a marker of
overall intestinal health as it takes into account both CD and VH. This trial included a total of 240 measures for each treatment, which is a considerable amount and therefore it should be noted that taking more measures to try and reduce associated error would have major time and cost implications. The inherent variability both in this study and other studies suggests that measuring VH and CD may not be an optimal approach to quantifying gut health.

This study showed that the greatest benefit of NCF to FCR occurred at the beginning of the trial, indicating there may be an optimum time for the supplementation of NCF to increase the efficiency of the birds. This may be due to the fact that the gut microflora of younger birds is more transient in nature and less established than in older birds and therefore more susceptible to colonisation by pathogenic bacteria. Therefore prebiotic intervention may shorten the time required to create a beneficial microflora population if it is offered early in life. However, similar studies have found variable early performance effects (Zhang et al., 2005; Iji et al., 2001; Sun et al., 2005; Midilli et al., 2008). In this study it was generally shown that NCF improved FCR over the starter and grower feeding phases. This suggests that the inclusion of NCF in the diet at both the starter and grower phase, increases the efficiency of the birds. This concurs with Zhang et al. (2005), however, other studies have shown no early response (Iji et al., 2001; Sun et al., 2005; Midilli et al., 2008).

It could be hypothesised that the improvements observed in FCR early on in the study when feeding NCF may have been due to an increase in absorptive area, due to an increase in lactobacilli and bifidobacterial populations and a reduction in pathogenic bacteria. This has been seen in other studies where improvements in gut morphology is associated with increased lactobacilli and bifidobacterial populations (Baurhoo, et al., 2009). However, improvements in gut health were not observed in this present study as histological measurements were only recorded at 6 weeks, when the microflora is already established with a population of beneficial bacteria. This means that birds...
on all treatment groups were not under any challenge and it was seen that NCF had no effect on
the performance in the last phase of the trial, which is consistent with the histological measurements
at 6 weeks.
There is no consensus on whether an increase in goblet cell numbers and area is considered an
improvement in bird health. Increasing the number and area of goblet cells is thought to increases
the volume of mucin stored in the GIT and possibly its production (Brümmer et al., 2010). Mucin
is essential for a number of brush border processes, including facilitating absorption of nutrients,
containing enzymes, lubrication and decreasing the binding and colonisation of pathogenic bacteria
to the intestine (Blomberg et al., 1993; Smirnov et al., 2004). Therefore an increase in the level of
mucin could have a beneficial effect on the first line of defence of the immune system (Baurhoo et
al., 2009) and the absorptive function of the gut. Contrarily, overproduction of mucin may result
in an negative effect, by increasing the mucus thickness on the GIT wall to a level that might
negatively affect the ability of nutrients to pass through to the gut epithelial to be absorbed
(Smirnov et al., 2004; Brummer, et al., 2010).
In this study, the number and area of goblet cells in the duodenum and jejunum were not affected
by the supplementation of NCF at 42 days. This would suggest that NCF has no effect on the mucin
profile of broilers in the duodenum in this study. This absence of response was also reported by
Castillo et al. (2008) and Yitbarek et al. (2012), however Baurhoo et al. (2007; 2009); Chee et al.
(2010); Morales-lopez et al. (2010) and Muthusamy et al. (2012) all found goblet cell numbers
were increased by yeast cell wall product supplementation. In the jejunum it was seen that NCF
increases the area of goblet cells and there was a trend for increasing goblet cell area per 165 μm
of villi, suggesting that NCF at 800g/t affects the mucin profile of broilers in the jejunum. Published
data on goblet cell area is very limited: Brummer et al. (2010) also found that the area of goblet
cells increased with supplementation of MOS. Insight into the mechanisms behind this goblet cell
response would be highly beneficial to understanding the effect of NFC supplementation on gut
health.

An increase in goblet cell area is thought to show that the storage capacity of the goblet cell for
mucin has increased (Smirnov et al., 2005). The increase in mucin storage may suggest that the
bird is more capable of forming a protective layer on the villi, thereby helping protect the intestine
from damage caused by enteropathogens if there was a challenge from pathogenic bacteria
(Smirnov et al., 2006; Brümmer et al., 2010). One suggested mechanism of MOS on mucin
production is through changing the gene expression of key genes through direct crosstalk between
beneficial intestinal microbes and goblet cells, (Mack et al., 1999; Freitas et al., 2003; Smirnov et
al., 2005 Uni and Smirnov 2006; Chee, 2008). The effects of NCF on the goblet cell area observed
in the current study agree with this suggestion in that goblet cell area increased with increased
supplementation level, but it is also possible that NCF is having a direct effect on mucin production
and subsequently increasing Bifidobacteria due to the increase in mucin production, as
bifidobacteria can produce enzymes allowing them to utilise and proliferate on mucin glycoproteins
(Katayama et al., 2005; Jung et al., 2008; Ruas-Madiedo et al., 2008).

CONCLUSION

Published research in this field appears to be highly variable, which may be due to differences in
the type of MOS product, experimental conditions, diet formulation, or health status of the birds.
This means that the mechanism of action of MOS and NCF and interactions with other nutritional
and production parameters are still not fully understood. In conclusion, under the conditions of
this trial, NCF is having an positive effect on performance in the starter and grower phases
indicating that the performance benefit of NCF could be age-dependent with younger birds
responding more than the older ones. Also, although there were no additional performance benefits
to feeding NCF for a longer period, goblet cell area in the jejunum was increased, suggesting that
birds fed NCF would have greater defences to pathogenic challenges due to higher levels of mucin.

ACKNOWLEDGMENTS

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DECLARATION OF INTEREST

This study was part of a PhD project that is part funded by Alltech. Peter Spring holds the role of
a consultant to Alltech and Jules Taylor-Pickard is currently employed by Alltech.

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2272.

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products: The effects on gut morphology and performance of broiler chickens. South African


Table 1. Composition of basal diet and calculated analysis of the basal diet

<table>
<thead>
<tr>
<th>Item</th>
<th>Starter</th>
<th>Grower</th>
<th>Finisher</th>
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<tr>
<td>Ingredients (%)</td>
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</tr>
<tr>
<td>Barley</td>
<td>10.60</td>
<td>8.46</td>
<td>7.23</td>
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<tr>
<td>Wheat</td>
<td>50.00</td>
<td>55.00</td>
<td>60.00</td>
</tr>
<tr>
<td>Soya ext hipro</td>
<td>26.00</td>
<td>23.00</td>
<td>19.00</td>
</tr>
<tr>
<td>Full fat soya bean meal</td>
<td>5.00</td>
<td>5.00</td>
<td>0.50</td>
</tr>
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</table>
L lysine HCL    0.31  0.26  0.25
DL methionine  0.38  0.35  0.33
L threonine    0.14  0.13  0.14
Soya oil       4.00  4.50  4.75
Limestone      1.25  1.25  1.25
Monocalcium phosphate  1.50  1.25  1.25
Salt           0.25  0.25  0.25
Sodium bicarbonate  0.15  0.15  0.15
Premix*        0.40  0.40  0.40

Calculated analysis

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<th>Attribute</th>
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<th>Cal 2</th>
<th>Cal 3</th>
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<td>ME MJ/kg</td>
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<td>13.00</td>
<td>13.20</td>
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<tr>
<td>CP %</td>
<td>21.80</td>
<td>20.60</td>
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<tr>
<td>Lys %</td>
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<td>1.16</td>
<td>1.13</td>
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<td>Met + Cys %</td>
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<td>Ca</td>
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<td>Available P</td>
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*Premix content (volume/kg diet): Mn 100mg, Zn 80mg, Fe 20mg, Cu 10mg, I 1mg, Mb 0.48mg, Se 0.2mg, Retinol 13.5mg, Cholecalciferol, 3mg, Tocopherol 25mg, Menadione 5.0mg, Thiamine 3mg, Riboflavin 10.0mg, Pantothenic acid 15mg, Pyridoxine 3.0mg, Niacin 60mg, Cobalamin 30µg, Folic acid 1.5mg, Biotin 125mg

Table 2. Effect of NCF in broiler diets on the average body weight (g/bird) and FCR

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Age (days)</th>
<th>Control</th>
<th>NCF 200g/t</th>
<th>NCF 400g/t</th>
<th>NCF 800g/t</th>
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<th>P value</th>
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<tr>
<td>Body wt</td>
<td>1</td>
<td>43.33</td>
<td>43.90</td>
<td>43.58</td>
<td>43.05</td>
<td>0.34</td>
<td>0.851</td>
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<tr>
<td>(g)</td>
<td>7</td>
<td>102.44</td>
<td>114.20</td>
<td>106.68</td>
<td>107.05</td>
<td>2.02</td>
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<td></td>
<td>Villus Height</td>
<td>NCF 200g/t</td>
<td>NCF 400g/t</td>
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<td>SEM</td>
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<td>Duodenum</td>
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<td></td>
<td>Control</td>
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<td>2791</td>
<td>2891</td>
<td>2704</td>
<td>52.6</td>
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(Differing superscript within one week denote means are significantly different at P ≤ 0.05)
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<tr>
<th>Attribute</th>
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<th>Jejunum</th>
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<tr>
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<td>209</td>
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<td>Crypt Depth</td>
<td>265</td>
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<td>Villus/Crypt Ratio</td>
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<td>210</td>
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<td></td>
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<td>0.47</td>
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<td>8.9</td>
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<td></td>
<td>0.17</td>
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Table 4. Effect of NCF on the goblet cell of the duodenum and jejunum(± s.e)
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<tr>
<th></th>
<th>59.2</th>
<th>69.4</th>
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<th>62.6</th>
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<tr>
<td>Nº of GC per 165µm</td>
<td>11.8</td>
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<td>GC Area per 165µm</td>
<td>683.1</td>
<td>747.0</td>
<td>664.4</td>
<td>719.3</td>
<td>23.6</td>
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Jejunum

<table>
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<th></th>
<th>67.6&lt;sup&gt;B&lt;/sup&gt;</th>
<th>68.3&lt;sup&gt;B&lt;/sup&gt;</th>
<th>73.8&lt;sup&gt;AB&lt;/sup&gt;</th>
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<tr>
<td>Nº of GC per 165µm</td>
<td>12.4</td>
<td>12.2</td>
<td>12.0</td>
<td>11.8</td>
<td>0.2</td>
<td>0.741</td>
</tr>
<tr>
<td>GC Area per 165µm</td>
<td>847.0</td>
<td>868.0</td>
<td>882.1</td>
<td>988.4</td>
<td>21.9</td>
<td>0.089</td>
</tr>
</tbody>
</table>

(Differing superscript within one week denote means are significantly different at P ≤ 0.05)