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Reflux of digesta and its implications for nutrient digestion and bird health

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Executive Summary

Four experiments were conducted to evaluate the occurrence and possible inductive effects of certain dietary ingredients on reflux in broiler and layer chickens. The first experiment, conducted on broiler chickens, was a preliminary trial, to investigate the occurrence of reflux in fasted and fed chickens. Analysis of Cr content clearly indicated the prevalence of reflux throughout the gastrointestinal tract (GIT) of fed or fasted broiler chickens between 3 and 5 weeks of age. The second experiment looked at the effects of dietary fibre on the occurrence of reflux. Four treatment groups of 24 birds were placed on different diets; a basal diet or the basal diet supplemented with 10% oat hulls or 4% carboxymethyl cellulose (CMC), and a commercial diet. The results confirmed the occurrence of reflux in fed and fasted, 3 and 5-week old chickens. In addition, oat supplementation appeared to induce reflux. In the third experiment the passage of a bacterial marker and Cr-EDTA in broiler chickens fed diets based on maize or wheat was investigated. The bacterial marker was an antibiotic-resistant strain of *E. coli.* Results showed that both the bacterial marker and Cr-EDTA were relocated by reflux. No significant differences were observed between diet groups and marker levels in the various sections of the GIT. The fourth experiment was similar in design to the third experiment except that 39-week old layers were used. The layers were given two weeks to adapt to the experimental diets. Chromium was detected in the gizzard of birds from all diet groups, with the lowest mean concentration, although not statistically significant, in birds given the maize diet. The antibiotic-resistant *E. coli* was present in the gizzard of birds on all diets but there was no significant difference between the dietary groups. Analysis of digesta from the duodenum showed that Cr was present in this section of the GIT for birds from all diet groups but there was no significant difference between the groups. The microbial marker was also detected in the duodenum of birds from all four diet groups. Analysis of variance revealed significant differences ($p=0.05$) between the maize diet group and both Wheat+enzyme and commercial diet groups, in terms of number of colonies. The digesta retrieved from the caeca of birds from all four diet groups contained Cr and the antibiotic-resistant *E. coli*. Analysis of variance did not reveal significant differences between the Cr levels or *E. coli* counts in birds on the maize and wheat dietary groups. The results of the four experiments conducted suggest that reflux occurs throughout the digestive tract of both fasted and fed chickens. Reflux appears to be a part of normal gut motility as well as a possible adaptive response to an absence of food, indicating that it serves as a way of extending the digestive process. Although not conclusive from the results of the studies, dietary ingredients may affect reflux, especially ingredients that increase the viscosity of the digesta in the lumen. In addition, microbial populations may also be relocated by reverse peristaltic contractions, with implications for bird health.

Contents

Background

Supply of nutrients to the growing broiler and laying chickens occurs through a complex series of interactions, which can be viewed on one hand as series of enzymatic reactions hydrolysing the macromolecules of the feedstuffs and transporting them into circulation. However, while this is happening the food is moving through the GIT and may be excreted before digestion and absorption are complete. The extent of such losses depends on the rate of passage which is affected by the nutrient composition of the diet as well as composition of microbial populations, harmful and beneficial, competing for nutrients in the lumen. The loss of nutrients through excretion could be slowed as a result of greater reflux of material from the hindgut into the proximal regions of the GIT, particularly with highly fibrous materials (Hetland et al., 2003). In turkeys, intestinal reflux is believed to enhance nutrient utilization (Basha and Duke, 1999). However, reflux of terminal intestinal digesta which could contain potentially harmful microorganisms is highly undesirable to the health of the chicken. Together this indicates that further study of the phenomenon of antiperistaltic movement of digesta is important in understanding of digestive physiology, and in improving use of nutrients in the chicken. The project was aimed at identifying the differences between key feed ingredients, with regards to their potential to induce or inhibit reflux. The implications of reflux on nutrient utilization and the health of the birds was also examined. This could enable the development of new varieties of crops and feed additives, which are capable of regulating reflux, to achieve the desired efficiency of utilization of diets and health of birds.

Objectives

- To demonstrate the existence of digesta reflux in broiler and laying chickens. This will provide an overview of the importance of the process of reflux at various intestinal sites and how this will influence nutrient utilization.
- To demonstrate the relative importance of the reflux phenomenon in poultry under practical conditions.

Methodology

Experiment 1

Ninety-six day-old Cobb broiler chickens were obtained from Baiada Hatchery, Kootingal, NSW, Australia. The birds were randomly assigned to four groups of 24 and placed in a 4-tiered metal brooder.

At 21d of age, 24 birds were selected randomly and deprived of food for two hours prior to the administration of 1 mL (approximately, 3.89×10^{-4} g/ml) Cr-EDTA marker via the cloaca using a syringe and crop needle. The purpose of feed deprivation was to try and ensure that the bird's GIT was relatively empty and was unlikely to defecate during or just after marker administration. If the marker was seen to be passed out by the animal, another 1 mL was administered. The 24 birds were then placed in individual metabolic cages and divided into two further groups of 12, one of which received food and water and the other group received water but no food.

The ME cages were set up so that any faeces passed by the bird fell straight into the collecting tray therefore making it difficult for the birds to ingest their faeces. Four birds (2 birds from the fed group and 2 from the starved group) were slaughtered (by cervical dislocation) every hour for the first five hours after administering the marker and digesta samples were taken from the crop, gizzard, duodenum, jejunum, ileum and caeca for analysis for the marker. A total of 20 birds were slaughtered by cervical dislocation on the day of marker administration and the final four slaughtered exactly twenty-four hours after the marker was administered. The same procedure was followed with the remaining group on day 35. All the birds were fed a commercial starter diet for the first two weeks and then a commercial finisher diet for the remainder of the trial.

The birds were monitored daily for feed intake and health throughout the trial. Any ill or injured birds were euthanased by cervical dislocation and the carcasses disposed of according to ethical regulations.

Once the digesta had been collected it was freeze-dried, and then ground using an Ika-a10, stainless steel, water-jacketed, cross-beater mill. The dry, ground samples were then digested using the technique described below in preparation for inductively coupled plasma (ICP) mineral analysis. The ICP yielded the concentration of Cr present in the digesta samples.

Procedure for preparation of Cr-EDTA for use as an indigestible marker

In an 800 mL beaker, 14.2 g of pure chromium trichloride ($CrCl_3 \cdot 6H_2O$) was dissolved in 200 mL of distilled water. Then 20g of the disodium salt of EDTA was dissolved in 300 mL of distilled water and added to the first solution. The combined solution was heated to boiling point with a few boiling chips. Once at boiling point, the solution was covered with a watch glass and left to boil gently for one hour. Over this time, the solution gradually assumed a deep violet color as the 1:1 complex of EDTA and Cr is formed. After heating, the excess EDTA was neutralized with 4 mL of a 1.0 M calcium chloride solution. The pH was then set to between 6 and 7 with small amounts of NaOH or HCL and then made up to 1L (Binnerts *et al.*, 1968).

Digesta mineral analysis – perchloric acid/hydrogen peroxide digestion technique

About 0.2 g (±0.01 g) of ground sample was weighed into a Schott bottle of known weight. Two mL of a 7:3 (v/v) mixture of $HClO_4$ (70%) and H_2O_2 (30%) was added to each tube and capped loosely. After pre-digestion for a minimum of 2 hours at room temperature, 1 mL of H_2O_2 was added and then the bottle was tightly sealed to allow the digest to heat under pressure in an oven set at 80^{o}C (±2^{o}C) for 30 minutes. The bottles were allowed to cool slightly (approximately 15 minutes) and a further 1 mL H_2O_2 was added. The cap was then placed on tightly and the mixture was allowed to digest for 1 hour at 80^{o}C. If further digestion of the sample was necessary, then digests with 1 mL aliquots of H_2O_2 for 30 minute intervals at 80^{o}C were carried out. The sample should was clear but no more than two additions of H_2O_2 were made at this stage. The samples were allowed to cool, made to volume or weight (original weight, bottle only plus 25g) using Milli-Q water and then mixed thoroughly. The samples were left for a minimum of 2 hours or overnight before filtering the silicate precipitate out of the sample using No. 1 Whatman filter paper. The samples were stored in glass vials with positive snap-on caps at 2^{o}C, to reduce absorption onto plastic and growth of microorganisms, prior to ICP analysis.

Between digest runs, all glassware was washed in tap water thoroughly, rinsed in de-ionised water and then soaked in 10% HCl for a minimum of 2 hours. Due to the limited number of replicates, and this being preliminary investigations, no statistical analysis was carried out on the data.

Experiment 2

Ninety-six day-old Cobb broiler chickens were obtained from the same hatchery as in experiment 1. The birds were randomly assigned to four groups of twenty-four and placed in their groups in a four-level battery brooder.

At 21d of age, the four groups were each randomly assigned a different diet of varying fibre content. One of the experimental diets was corn-sorghum-based and prepared at the University of New England; two other diets were the basal diets supplemented with oat hulls or carboxymethyl cellulose (CMC), a highly viscous compound. The fourth was a commercial diet.

On day 28, twelve birds from each group were placed in individual ME cages. Food was not available to any of the birds during this time but water was provided. After two hours of food deprivation, each group was divided into a further two sub-groups, one with access to food and water and the other with only access to water. Immediately after this, 1 mL of Cr-EDTA marker was administered to all birds via the cloaca. One hour after the marker was administered four birds from each diet group (two starved, two fed) were slaughtered (by cervical dislocation) and digesta samples taken from the crop, gizzard, duodenum, jejunum, ileum and caeca to analyse for the marker. This was repeated five hours and twenty-four hours after marker administration. The same procedure was repeated at 35 days post-hatch, on the remaining forty-eight birds. Due to the limited number of replicates, and this being preliminary investigations, no statistical analysis was carried out on the data.

Experiments 3 and 4

In experiment 3, 72 day-old broiler chicks were randomly divided into six groups. The groups were placed on diets based on maize (M), wheat (W), wheat supplemented with a microbial enzyme (W+e) and a commercial diet (C). A fifth group was fed a commercial diet and both markers were administered at 28 days of age via the crop while Group 6 was fed a commercial diet and only the Cr-EDTA marker was administered via the cloaca at 28 days old. Each group contained 12 birds; 6 replicates per group, 2 birds per replicate, therefore, each ME cage contained 2 birds. At 28 days of age all birds were deprived of feed for 4 hours prior to the administration of the two markers (Cr-EDTA and antibiotic-resistant *E. coli*), then returned to their food. In the previous two experiments the birds were deprived for only 2 hours prior to marker administration, the reason for extending this time was to try and prevent the birds defecating during administration, which had proved to be a problem previously.

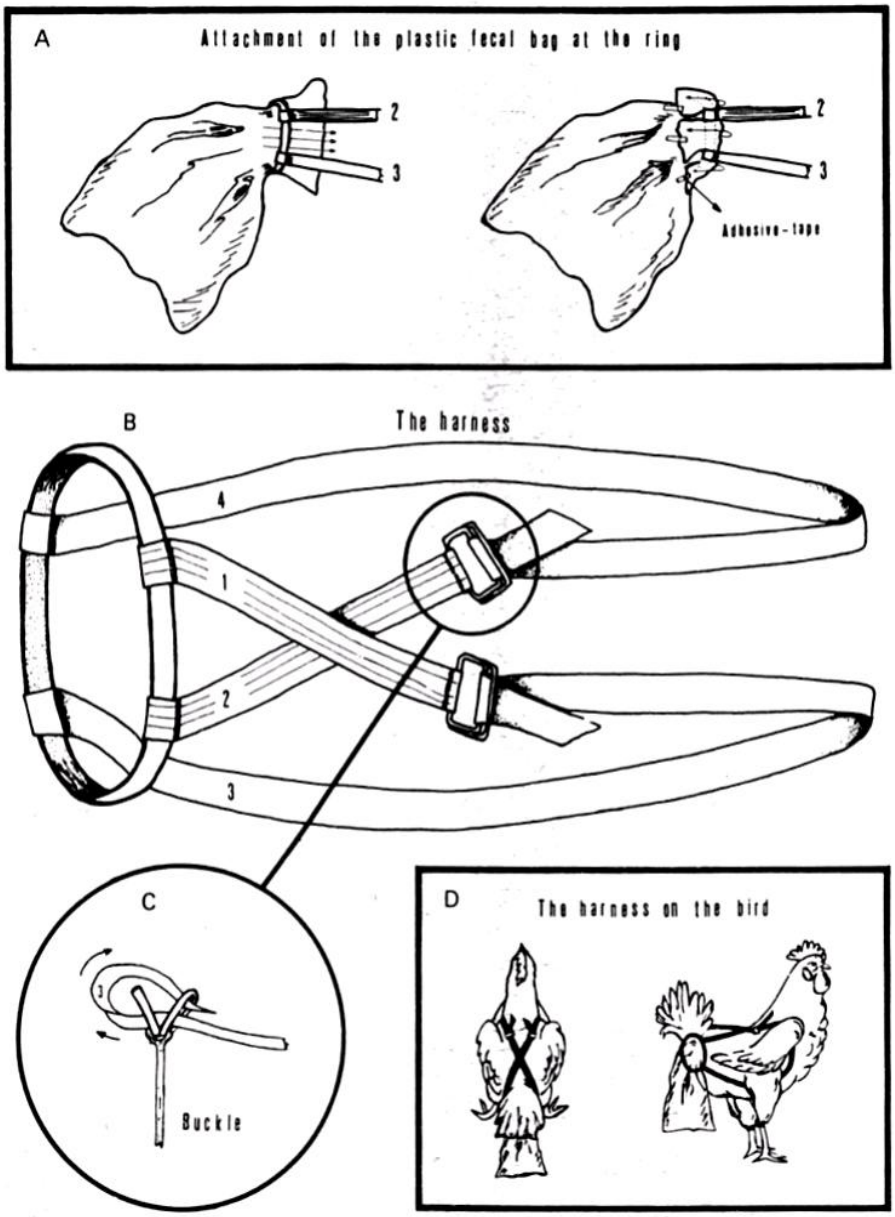


Figure 1: Schematic illustration of the harness (Almeida and Baptista, 1984). A similar harness was used in the present study.

After the markers were administered a harness was placed on each of the birds to collect the faeces. This was to help minimise the chances of the birds ingesting their own faeces and therefore the markers. In the previous experiments, at times, it was observed that certain birds had faeces on their beaks. Although it was

unlikely that they actually ingested the faeces (the results support this) when dealing with a microbial marker it is possible that presence of faeces anywhere around the mouth may affect the results. The design of the harness was a variation of one used in a study carried out by Almeida and Baptista (1984). The harness consisted of a plastic ring (approximately 50mm in diameter) with four ribbons attached to it (Figure 1).

At exactly 24 hours, post-administration (accuracy was achieved by staggering the marker administration for each replicate to allow for enough time to sample both birds the following day), all the birds from each treatment group were slaughtered (by carbon dioxide asphyxiation) and digesta samples were taken from the gizzard, duodenum, jejunum, ileum, caeca and colon.

Approximately one gram of digesta from each section of the GIT was added to a McCartney bottle containing 10 mL of peptone water. These underwent multiple dilutions (gizzard, duodenum, jejunum and ileum underwent three further dilutions while four dilutions were done for the caeca and colon samples). The last two dilutions were plated out on antibiotic and non-antibiotic-resistant McConkey agar plates, to analyse for the presence of the resistant *E. coli.* in the digesta samples. The remaining digesta was freeze-dried, ground and then analysed for Cr content. The fourth experiment was similar to the third in details except that hens in production were used in place of broiler chickens and reflux was assessed only 24 hours after marker administration, following death by CO_2 asphyxiation.

Results

Experiment 1

At both 3 and 5 weeks of age, one hour after marker administration, Cr was detected as far up the GIT as the crop in the fed groups (Figures 2 and 3). In the fasted group Cr was detected as far up as the gizzard. Chromium concentrations were log-transformed reduce the effects of outlying values.

After one hour, Cr was detected in all sections of the GIT except for the 3-week old group in which none was detected in the duodenum. In both fasted and fed groups at both ages, the majority of the marker remained in the caeca. In the 3-week old, fasted group high levels of Cr were observed in the gizzard equivalent to the levels found in the corresponding caecal samples.

Two hours post-marker administration, Cr was not found in the crop in the fasted and fed birds of 3 weeks of age or in the 5-week old, fasted birds (data not shown). Cr was detected in both fasted and fed birds in all the other regions of the GIT at 5 weeks-old, with the levels remaining the highest in the caeca. In the 3-week old birds, in addition to the crop, Cr was absent in the jejunum and ileum for fasted groups, but in the gizzard, duodenum and caeca Cr levels were higher than in the fed group.

Three hours after marker administration, in the 3-week old birds, Cr was present in all sections of the GIT for the fed group, with the highest levels being observed in the caeca and the crop. For the fasted group, Cr was found in the gizzard (at higher levels than in the corresponding fed group) and at low levels in the jejunum and decreasing levels in the caeca.

In the 5-week old birds, there was little difference between the levels of Cr observed in the fed and fasted groups, except for the absence of Cr in the crop of the fasted group. Chromium levels were higher in the gizzard, duodenum, jejunum and caeca in the fasted group than in the fed group.

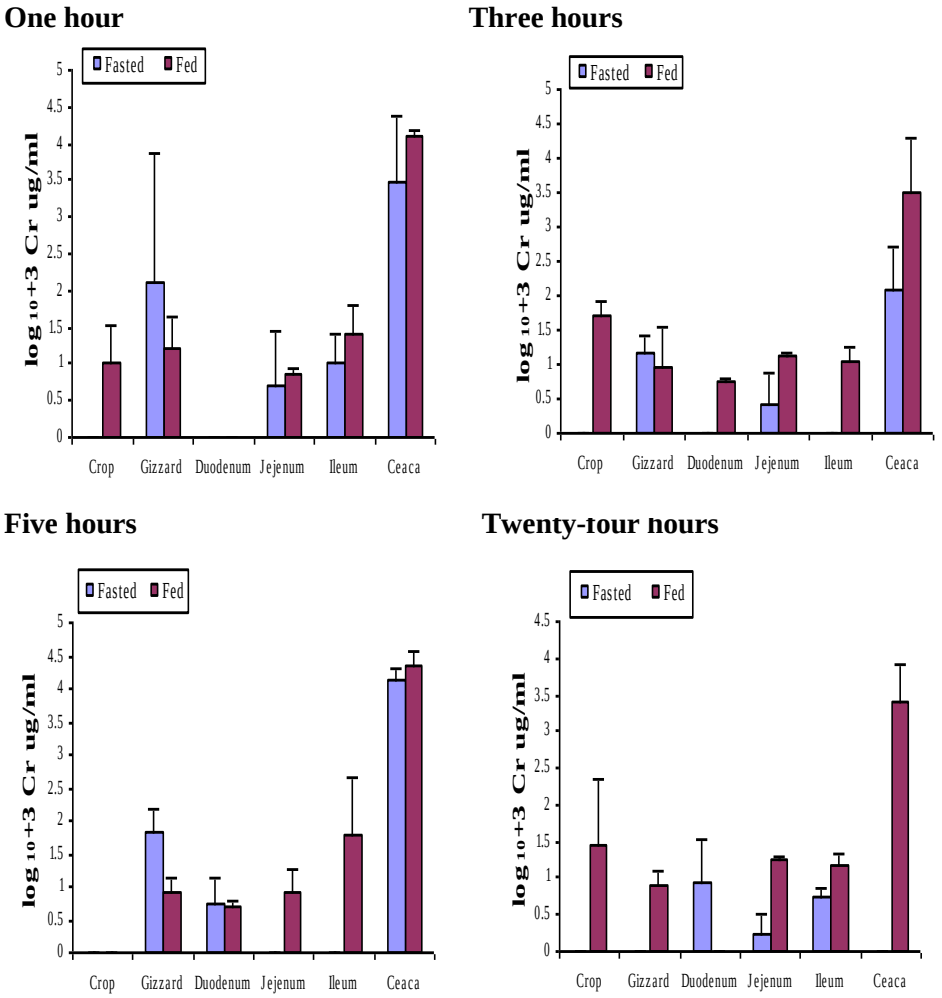


Figure 2: Concentration of Cr in different regions of the GIT at different times in 3-week old broiler chicks.

In the fasted birds, Cr was absent in the jejunum and ileum, 4 hours after administration in both the 3- and 5-week old birds. Chromium was present in other regions, except in the crop of fasted birds (both 3- and 5-week old). Cr levels were higher for fasted birds in the gizzard and duodenum at both ages. Levels in the caeca remained the highest for all birds.

The ileum and jejunum of both fed and fasted 3-week old birds were devoid of marker, five hours into observation. The marker was also absent in the crop, jejunum and ileum of the 5-week old fasted birds. In general, fed birds showed more evidence of reflux than fasted birds.

Twenty-four hours after the marker was administered Cr levels remained highest in the caeca for most birds, with levels only slightly less than those observed 1 hour post-administration. In fed birds, Cr was present at all regions with the exception of 3-week old fed birds where Cr was absent in the duodenum. For fasted birds, in both age groups, some regions of the GIT were devoid of Cr.

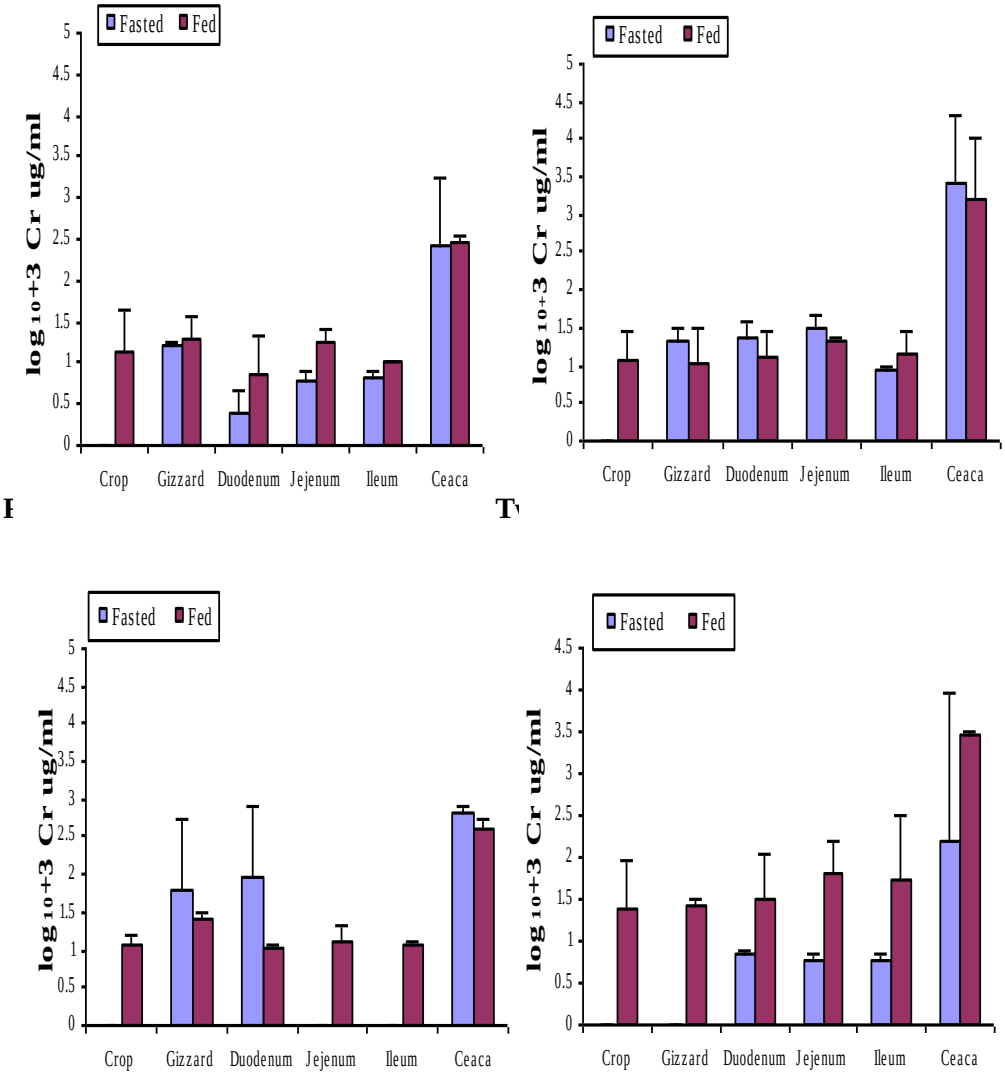


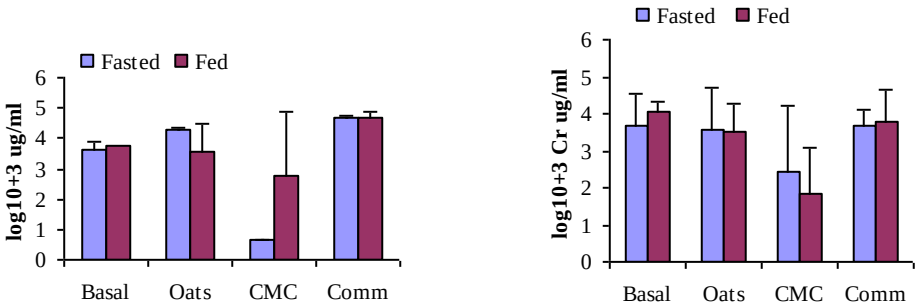
Figure 3: Concentration of Cr in different regions of the GIT at different times in 5-week old broiler chicks.

Experiment 2

Figure 4 shows the concentration of Cr in the caeca of fasted and fed birds, at both ages. In all diet groups, at 1 hour post-marker administration, the marker was detected in the caeca. The Cr levels in the caeca of the fasted, 3-week old birds from the CMC diet group was the only Cr detected in any section of the GIT of fasted birds from this group, 1 hour into the trial.

After 5 hours, Cr was present in the caeca of fasted and fed, 3-week old birds, from the basal, oat hull and commercial diet. The marker was present in the caeca of only fed, 3-week old birds from the CMC diet group. The relatively small amounts retrieved and the complete absence of Cr in the fasted birds is likely due to the large amounts of Cr detected in the two sections proximal to the caeca. In the 5-week old birds, Cr was detected in the caeca of fasted and fed birds from all four diet groups. After 24 hours, Cr was present in the caeca of fasted and fed birds, of both ages, from all four diet groups.

1 hour



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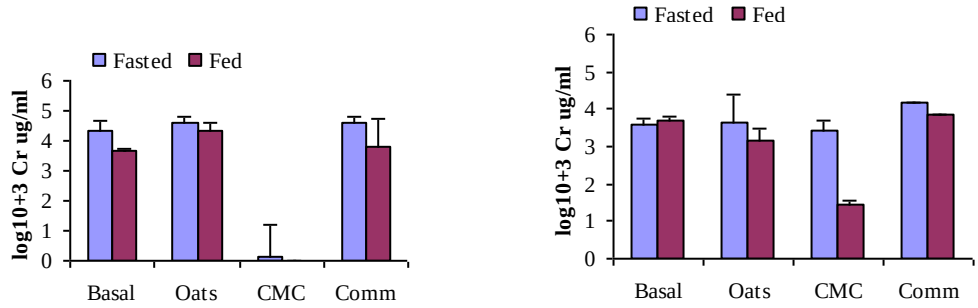


24 hours

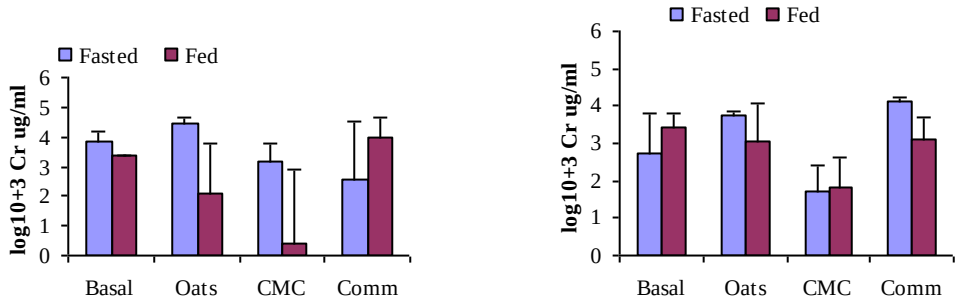
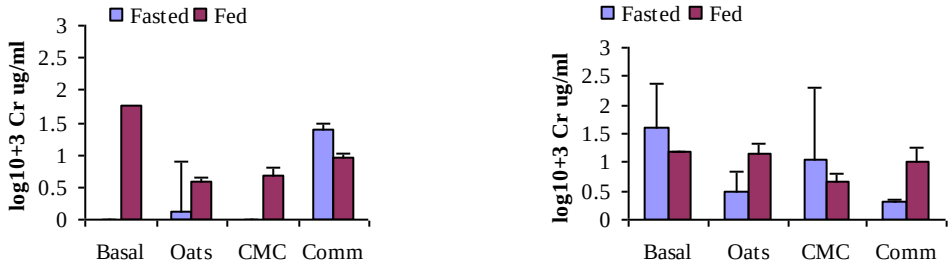


Figure 4: Concentration of Cr in the caeca at different times post-marker administration, in fed or fasted birds reared on different diets at 3 (left) and 5 (right) weeks of age.

Chromium was present in the ileum of fasted and fed, 3-week old birds, from the basal, oat hull and commercial diet groups, 1 hour after administration of the marker (data not shown). The marker was detected only in the ileum of the fed 3-week old birds from the CMC diet group. At 5 weeks of age, at the same point in time, Cr was retrieved from the ileum of fasted and fed birds from all four diet groups.

After 5 hours, Cr was recovered from the ileum of fasted and fed, 3-week old birds, from the oat hull, CMC and commercial diet groups. The marker was present in only the ileum of fed, 3-week old birds, from the basal diet group. At 5 weeks of age, Cr was found in the ileum of the fasted and fed birds from the oat hull and CMC group. Chromium was detected in only the fed, 5-week old birds from the basal and commercial diet groups.

1 hour



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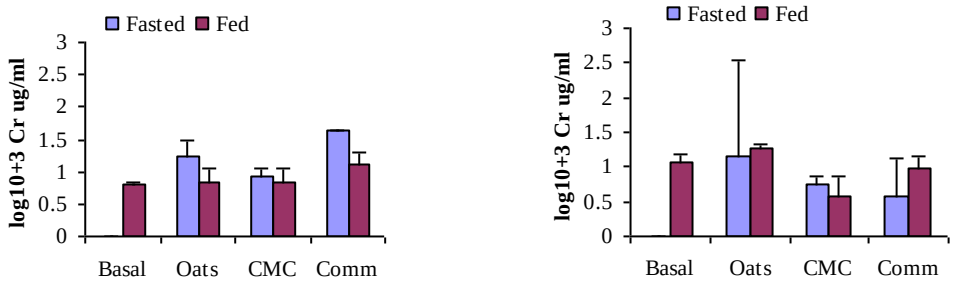

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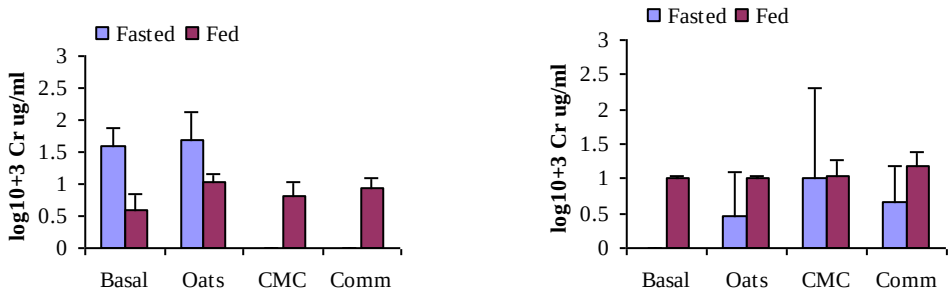


Figure 5: Concentration of Cr in the jejunum at different times post marker administration, in fed or fasted birds reared on different diets at 3 (left) and 5 (right) weeks of age.

Twenty-four hours after marker administration, Cr was recovered from the ileum of fasted and fed, 3-week old birds, on the oat hull, CMC and commercial diets. Chromium was present only in the ileum of fed, 3-week old birds from the basal diet group. At 5 weeks of age, at the same point of assessment, Cr was present in the ileum of fasted and fed birds, from all four diet groups.

As shown in Figure 5, Cr was found in the jejunum of fasted and fed, 3-week old birds, from the oat hull and commercial diet, 1 hour into assessment. The marker was present in the jejunum of only fed, 3-week old birds from the basal and CMC diet groups at this point in the trial. At 5 weeks of age, Cr was detected in the jejunum of fasted and fed birds from all four diets, 1 hour after administration of the marker

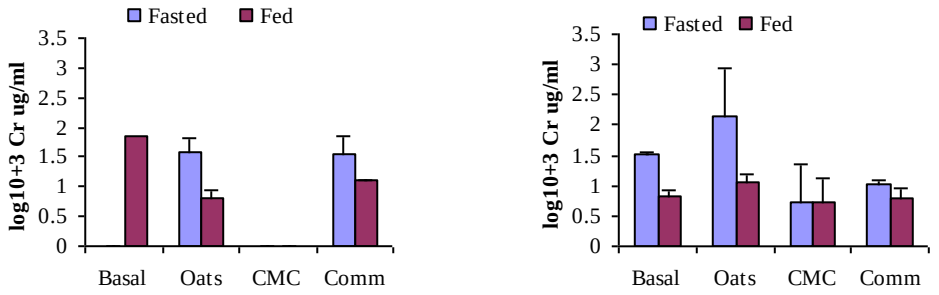
Five hours after marker administration, Cr was present in the jejunum of fasted and fed, 3-week old birds, from the oat hull, CMC and commercial diet groups. The marker was detected only in the jejunum of the fed, 3-week old birds from the basal diet group. In the 5-week old birds, Cr was recovered from the jejunum in fasted and fed states, on the commercial diet and diets supplemented with oat hulls and CMC. Chromium was present in only the fed 5-week old birds from the basal diet group. By 24 hours post-marker administration, Cr was detected in the jejunum of fasted and fed 3-week old, birds form the basal and oat hull-supplemented diets. The marker was present in only the fed 3-week old birds from the CMC and commercial diet groups. At 5 weeks of age, Cr was present in the jejunum of fasted and fed birds from the

oat hull, CMC and commercial diet groups. Chromium was present in only jejunum the fed 5-week old birds from the basal diet group.

It should be noted, that samples taken from the fed, 3-week old birds, from the basal diet group, were pooled and therefore standard deviations could not be calculated. In the duodenum, the marker was detected at this region in fasted and fed 3-week old birds, from the basal and oat hull diet groups, 1 hour after administration (data not shown). No Cr was present in any of the 3-week old birds raised on the CMC diet. Chromium was also retrieved from the duodenum of fasted 3-week old birds, raised on the commercial diet. At 5 weeks of age, at the same point in the trial, Cr was detected in the duodenum of fasted and fed birds from both the oat and CMC diet groups. The marker was present in the fasted 5-week old birds from the basal diet but not in the fed birds.

After 5 hours, Cr was detected in the duodenum of fasted and fed birds, from the oat hull and commercial diet groups. No Cr was retrieved from the duodenum of fasted or fed birds from the CMC diet group. Chromium was present in the fasted 3-week old birds from the basal diet group. At 5 weeks of age, at the same point in time Cr was present in the duodenum of both fasted and fed birds from all four diet groups.

1 hour



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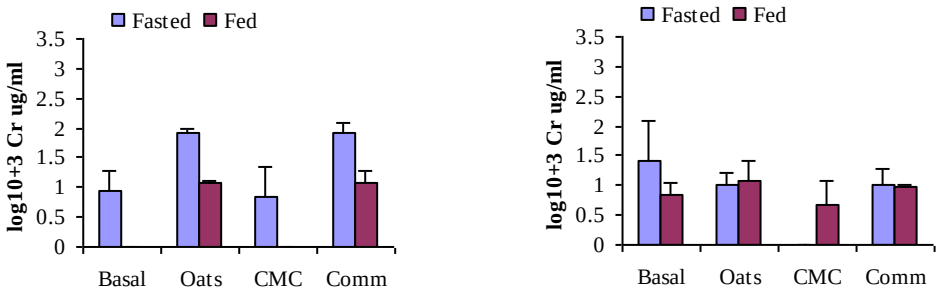

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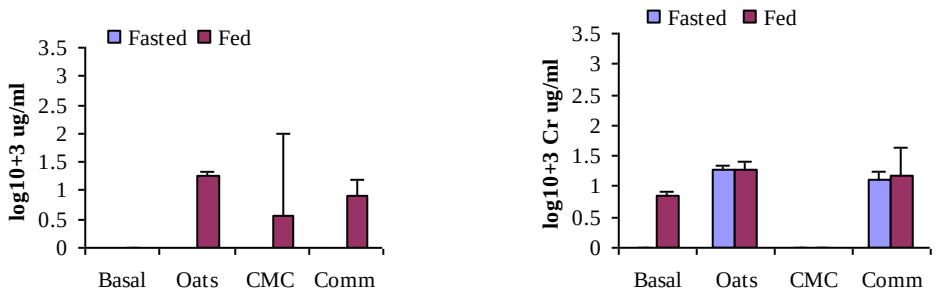
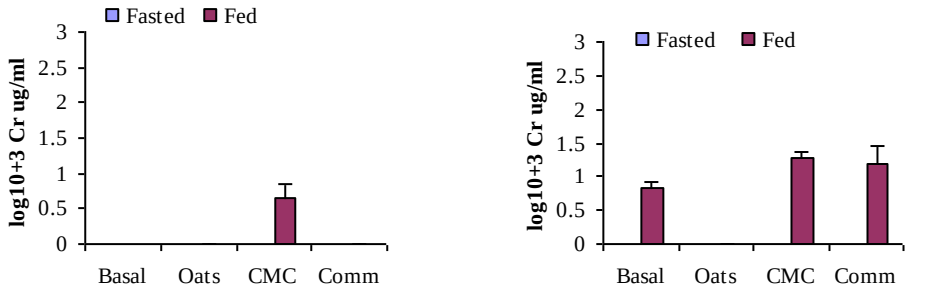


Figure 6: Concentration of Cr in the gizzard at different times post-marker administration, in fed or fasted birds reared on different diets at 3 (left) and 5 weeks of age.

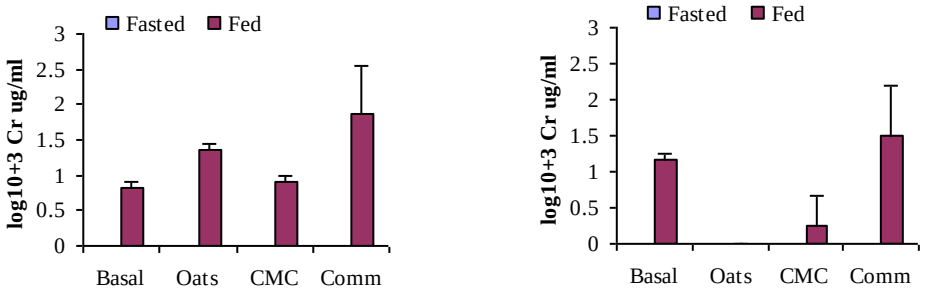
Twenty-four hours after the administration of the marker, Cr was present in the duodenum from fasted and fed, 3-week old, birds from the oat hull diet group. Chromium was retrieved from the duodenum of only fasted, 3-week old birds from the basal and CMC diet groups. Chromium was present in the duodenum of only fed, 3-week old birds from the commercial diet group. At the same point in time, Cr was found in the duodenum of fasted and fed birds on the commercial or oat hull and CMC-supplemented diets. The marker was only present in the duodenum of fasted 5 weeks-old birds, from the basal diet group.

Chromium was detected in the gizzard of 3-week old, fasted and fed birds, raised on the oat hull and commercial diets, 1 hour after marker administration (Figure 6). The marker was not detected in the gizzard of 3-week old, fasted birds, raised on the basal diet but was present in the fed birds from the same diet group, 1 hour into the evaluation. Chromium was not present in the gizzard of either the 3-week old, fasted or fed birds, raised on the CMC diet. For the 5-week old birds, at the same point in the trial, Cr was present in the gizzard of all birds, fasted and fed, from all four diet groups.

1 hour



5 hours



24 hours

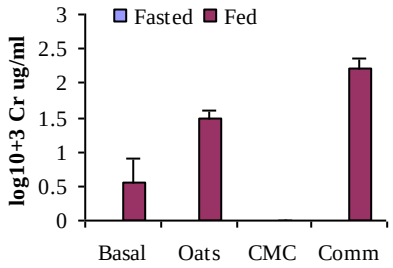


Figure 7: Concentration of Cr in the crop at different times post-marker administration, in fed or fasted birds reared on different diets at 3 (left) and 5 weeks of age. No Cr was recovered from the crops of any 5-week old chickens, 24 hours after the marker was administered to the birds.

At 5 hours post-marker administration, Cr was detected in the gizzard of 3-week old, fasted birds, raised on the basal diet. Chromium was present in the gizzard of the fasted and fed, 3-week old birds, from both the oat hull and commercial diet groups. The soluble marker was found in the gizzard of the fasted 3-week old birds from the CMC diet group but not in the fed birds. In the 5-week old birds, at the same point in the trial, Cr

was present in the gizzard of fasted and fed birds, from the basal, oat hull and commercial diet groups but only in the fed birds from the CMC diet group.

Twenty-four hours after the administration of marker, Cr was detected in only the gizzards of fed, 3-week old birds from the oat hull, CMC and commercial diet groups. For the 5-week old birds at the same point in time, Cr was retrieved from the gizzard of fed and fasted birds from the oat hull and commercial diet groups. The marker was present in the fed, 5-week old birds from the basal diet. No Cr was detected in any of the 5-week old birds from the CMC diet group.

As shown in Figure 7, Cr was not detected in the crop of the fasted birds at any point in the trial. At 3-weeks old, Cr was detected only in the crop of the fed birds on the CMC diet, 1 hour after administration. At 5-weeks old, 1 hour after administration of the marker, Cr was present in the crop of the fed birds raised on the basal, CMC and commercial diets but not in the birds raised on the oat hull diet. At this time, Cr levels were highest in the crop of the fed birds raised on the CMC diet.

At 5 hours post-marker administration, Cr was present in the crop of 3-week old fed birds, raised on the four different diets. Chromium levels were highest in the crop of fed birds raised on the commercial diet. At 5 weeks of age, 5 hours into the assessment, Cr was absent in the fed birds raised on the oat hull diet but was present in the crops of the fed birds raised on the other three diets. After 24 hours, Cr was detected in the crop of the fed 3-week old chickens, raised on the basal, oat hull and commercial diets but was absent in the birds raised on the CMC diet.

Experiment 3

The digesta retrieved from the caeca of birds from all four diet groups contained Cr. Analysis of variance did not reveal statistically significant differences between the levels found in the maize and wheat dietary groups (Figure 8). Colonies of the antibiotic-resistant *E. coli*. were present in the caeca of birds from all four diet groups, although there was no significant difference between the diets in terms of colony counts.

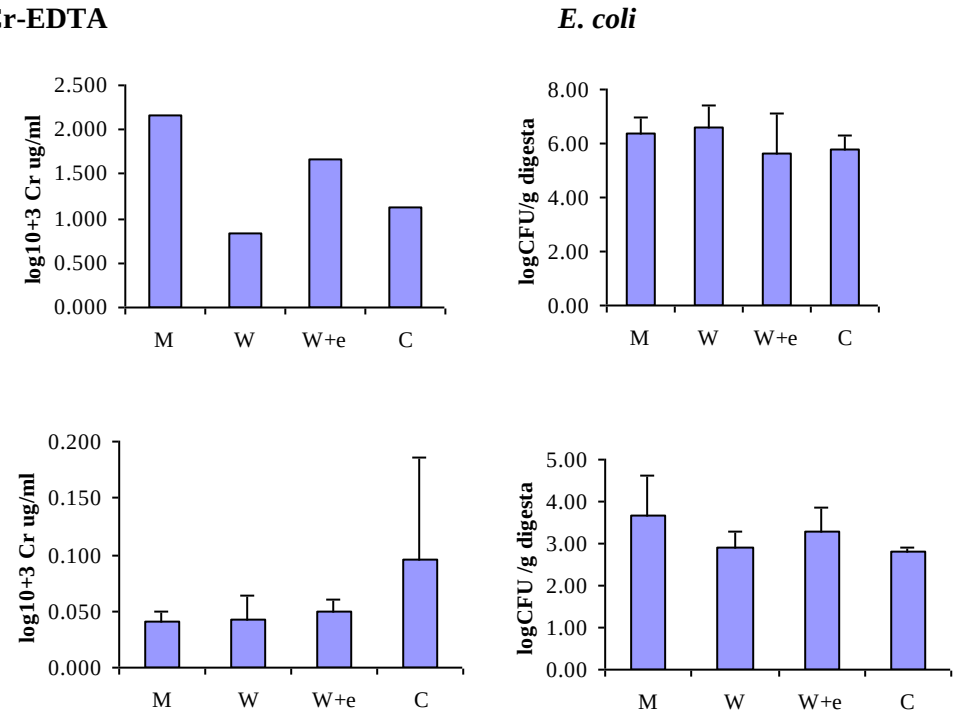


Figure 8: Cr-EDTA levels, and E. coli counts, detected in the caeca (top panel) and jejunum of birds on the four different diets; Maize (M), Wheat (W), Wheat and enzyme (W+e) and Commercial (C).

Although not shown, Cr was present in the ileum of birds from all four dietary groups. Although not statistically significant, the Cr levels in the ileum of birds from the maize and commercial diet groups were very low, almost nil.

The antibiotic-resistant *E. coli* was found to be present in the ileum of birds from all four diet groups, as with the Cr marker, there was no significant difference between the diet groups. Chromium was detected in the jejunum of birds from all four diet groups but there was no significant difference between the groups (Figure 7). The bacteria marker was lower in chicks on the commercial diet than on the other diets, but not significantly so. The marker was also detected in the jejunum of birds from all four dietary groups.

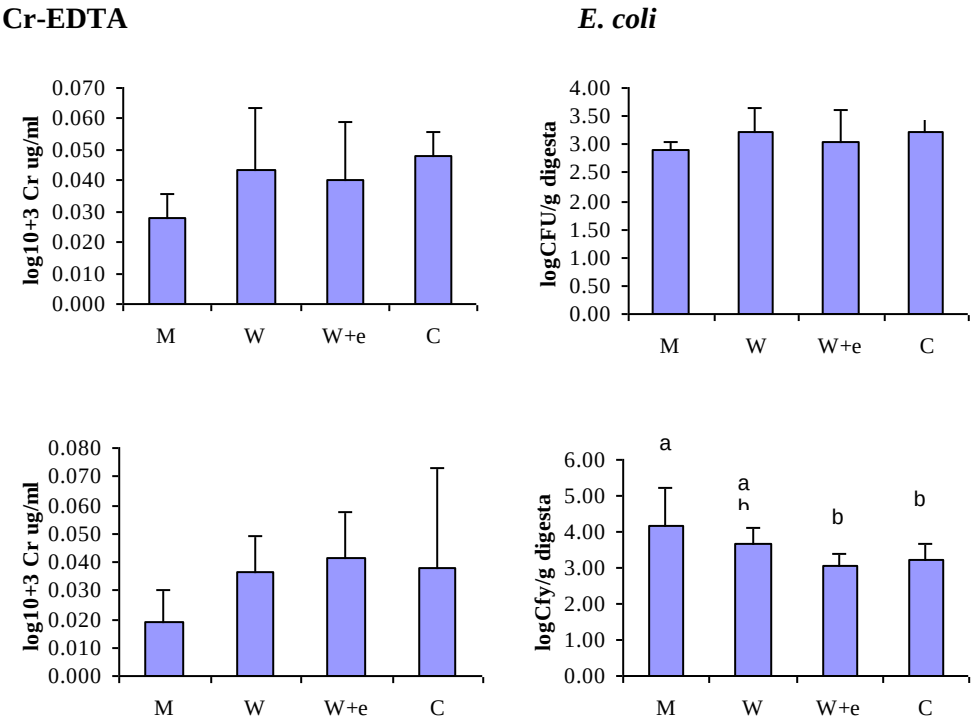


Figure 8: Cr-EDTA levels, and E. coli counts, detected in the duodenum (top panel) and gizzard of birds on the four different diets; Maize (M), Wheat (W), Wheat and enzyme (W+e) and Commercial (C).

Analysis of digesta from the duodenum showed that Cr was present in this section of the GIT for birds from all four diet groups but there was no significant difference between the groups (Figure 8). The microbial marker was also detected in the duodenum of birds from all four diet groups, as shown in Figure 8. Analysis of variance revealed significant differences ($P<0.05$) between the maize diet group and both W+e and commercial diet groups, in terms of number of colonies and diet.

Chromium was detected in the gizzard of birds from all four diet groups, as shown in Figure 8, with higher concentration in birds from the maize and commercial diets. The antibiotic-resistant *E. coli* was found to be present in the gizzard of birds on all four diets but there was no significant difference between the dietary groups.

Experiment 4

Cr was detected in the gizzards of layers from all four diet groups that had received the markers via the cloaca (Table 1). The Cr levels in the maize group were significantly different ($P<0.05$) from the levels detected in the gizzards of the wheat, wheat+enzyme and commercial diet groups.

The Cr marker was detected in the duodenum of birds from all four diet groups. No significant differences were observed in the amount of Cr between the diet groups, with all four diet groups showing similar levels of Cr. Chromium was found to be present in the ileum and caeca of layers from all four groups.

Table 1. Mean Cr-EDTA levels (Cr µg/ml) and *E. coli* (logCFU/g digesta) detected in the gizzard, duodenum and caecum of birds on the four dietary treatments.

	Commercial	Maize	Wheat	Wheat+enzyme	SEM
Cr-EDTA					
Gizzard	0.048	0.028	0.043	0.040	0.009
Duodenum	0.038	0.019	0.037	0.041	0.081
Caecum	1.133	2.170	0.844	1.658	0.564
E. coli					
Gizzard	3.238	2.907	3.211	3.051	0.256
Duodenum	3.221^{b}	4.158^{a}	3.671^{ab}	3.036^{b}	0.370
Caecum	5.862	6.408	6.567	5.839	0.531

a,b – Mean values on the same row with unlike superscripts are significantly different ($P<0.05$).

As shown in Table 1, the population of the indicator bacteria was significantly affected by diet, at most sites investigated. In all the regions assessed, the lowest populations of this marker were in diets based on maize. At the colon, close to the site of administration, both wheat diets and the commercial diet tended to increase the population of the marker, and the organism appeared to be more favourably refluxed to the proximal regions of the GIT in chickens raised on these diets too. The population of coliform bacteria, which was assessed alongside that of the marker was also lower in birds on the maize-based diets than in those on the other diets.

Discussion of Results

The results from this study, particularly from the first two experiments, clearly indicate the prevalence of reflux throughout the GIT of broiler chickens between 3 and 5 weeks of age. All birds were deprived of food 2 hours before the marker was administered via the cloaca so, in effect, the fasted birds had essentially been fasted for 3 hours when the first observations were made. According to previous studies carried out by Clench and Mathias (1992) and Jimenez *et al.* (1994), fasting acts as a trigger for antiperistaltic activity while extended periods of fasting – upwards of 4 hours - results in what they recognised as a rhythmic oscillating complex (ROC). The present study did not employ techniques to study myoelectric pulses, but the results concur with the idea that fasting induces reflux and would explain why Cr was present in the gizzards of birds from both feeding programs – the fed birds had also been deprived of food prior to the administration of marker. The results obtained in the present study also revealed the absence of marker in the crop of fasted birds. No reason could be adduced for this occurrence but it would appear that the magnitude of antiperistaltic movements would be aided by the presence of some food material in the upper part of the GIT.

The steady rise of Cr levels in the gizzard, and the subsequent peak at 4 hours, is more noticeable in fasted birds, and is likely a reaction to prolonged fasting. The high levels may be an indication of an ROC, suggested to occur after 4-6 hours of fasting and characterised by re-stimulating fed state motility. Peristalsis rather than reverse-peristalsis would be the expected norm for the fed state which might explain why after the peak at 4 hours, post-marker administration, Cr levels petered out until the marker was absent after 24 hours.

It has been suggested by Sklan *et al.* (1978) that gastro-duodenal refluxes are a normal part of digestive motility and this may be indeed the case, but the observed ROCs by Clench and Mathias (1992) resulted in an increase in the frequency of reverse peristaltic motility in the upper digestive tract that is not characteristic of normal motility. In general, Cr levels were higher in the gizzard and duodenum of fasted birds at both ages until about 4 hours into the observation. These comparatively high levels of Cr could be an indication of the

increased frequency of the gastro-duodenal reflux in reaction to an extended period without access to feed. The gastro-duodenal reflux, in light of these results, may be the preferred anti-peristaltic reaction to fasting. In addition, in most regions of the GIT over the time period studied, refluxed material (*i.e.,* Cr level) appeared to drain out of each section quicker than in fed birds. This further supports the idea that, for fasted birds, reflux may be an exhausting effort to recycle food already present in the GIT.

The high levels of Cr in the caeca are not surprising, since the marker was injected at a point just beyond the colonic sphincter. Colonic-caecal reflux is well known and is suggested to serve as a method for recovering nitrogen from urea. The levels observed especially in the 5-week old birds, suggest initial increasing levels (probably due to Cr entering via the colonic-caecal reflux), then levels decrease (due to reflux towards more proximal areas in the GIT) and finally a slight increase in levels again (material flowing distally under normal peristalsis).

In general, the amount of Cr present in the GIT decreased with time. In addition to this there were instances of Cr levels being high in one or two proximal sections - the crop, gizzard or duodenum- and then very low or absent in the adjacent distal section. This suggests that digesta, containing the soluble marker, has been pushed up and out of one section completely and into another.

Apart from supporting the results of the first experiment, the second trial aimed to make a preliminary investigation into the effects of feed ingredients on reflux, in particular insoluble fibre. Hetland *et al.* (2003) recorded the occurrence of reflux when birds had access to wood shavings. One of the diets contained 10% oat hulls but the results did not seem to be too different from those obtained from the birds raised on the basal diet (corn and sorghum) or the commercial diet. The birds fed the oat hull-supplemented diet throughout the trial period did, however, show the presence of Cr in the most proximal sections at higher levels and more frequently, 24 hours after the administration of marker. This suggests that insoluble fibre induces reflux but this will have to be studied in more detail.

One of the diets contained 4% CMC, a soluble fibre, and the birds fed on this diet excreted, in vast quantities, wet and sticky faeces. The volume of faeces excreted by these birds appeared to exceed that of the other diet groups. The extent of excretion gives some indication of how the diet was affecting them in terms of gastric motility. With a highly insoluble fibre diet, a fast rate of digesta passage is to be expected and it is thought that reflux occurs as an attempt to retain the digesta in the gut for a longer period of time. Most of the fibre in the CMC diet was water-soluble, therefore a slower rate of passage would be expected because of the viscous nature of the digesta. This does not agree with what was observed in regards to the bird's excretion patterns. It may be speculated that when viscous polysaccharides, such as CMC adhere to the gut walls the lumen is narrowed. This can accelerate the movement of liquid digesta refluxing up into the upper section of the gut. Unfortunately, a solid phase marker was not used in that study, which may have confirmed this hypothesis.

The third and fourth experiments investigated diets that were close to practical or practical in nature. The results of these experiments confirmed the findings in the previous two studies, with regards to the reverse passage of the soluble marker, Cr-EDTA. Due to the increased number of replicates included in the design of this trial, it is possible to state, with conviction, that reflux occurs from the posterior end all the way along the GIT to the anterior sections.

The two experiments also substantiate the hypothesis that bacterial communities can be relocated to proximal sites of the GIT by reverse peristalsis. The antibiotic resistant strain of *E. coli* used in the experiment, was recovered from the gizzard of birds from all four diets, indicating that the bacterium was able to travel the whole length of the GIT when introduced through the cloaca. The implications of this finding could be considerable, especially when related to the relocation of harmful, disease-causing microbes like *C. perfringens* to areas in the upper tract.

The harness used in the third experiment was originally designed by Almeida and Baptista (1984), as a way of collecting faeces. In this trial it served as a way of preventing the chickens from ingesting their own faeces. Although it appeared to work well enough, the harnesses proved rather difficult to put on the chickens and some time had to be repeatedly re-fitted to ensure the chickens did not try and pull them off

with their beaks. In addition, when the bag became full it tended to pull the harness out of position meaning regular checks had to made throughout the twenty-four hours to ensure the harness ring was sitting over the cloaca. If this method was to be used again, some modifications would have to be made to the harness to rectify these problems.

The other purpose of experiments 3 and 4 was to investigate the effect that diet had on retrograde movement of digesta in the GIT. Maize, wheat, and microbial enzyme-supplemented wheat-based diet were compared to a commercial diet. It was assumed that the main factor to affect digesta motility in the small intestine is digesta viscosity. Wheat as with other temperate cereals, like barley and rye, contains pentosans; these NSPs increase the viscosity of digesta by binding large quantities of water within the GIT and, consequently, interfere with digestion and absorption (Choct and Annison, 1992). The increase in viscosity of the digesta reduces physical mixing and transport of products to and from the brush-border membrane. Addition of exogenous enzymes (such as the commercial glycanase product Avizyme 1500) to wheat-based diets reduces the viscosity of the digesta through partial depolymerisation of the NSPs, resulting in an increase in digestibility and absorption (Choct *et al.,* 1996). In hindsight, Avizyme 1300 would have been a preferred supplement to the wheat-based diet. Maize is considered to be an excellent feedstuff for poultry mainly because it contains very low amounts of water soluble NSPs and therefore does not result in highly viscous digesta (Choct *et al.*, 1995).

In these experiments, it can be assumed that the apparent intestinal viscosities of the maize and enzyme-supplemented wheat-based diets were similar while the wheat control diet would result in a more viscous digesta, particularly in the jejunum and ileum. Consequently, one would expect to see significant differences in the levels of the two markers in the small intestine for the maize and wheat diets. However, this was not the case; significant differences were not observed between the marker levels in the maize and wheat diet in the experiment on broilers (experiment 3) but in the experiment on layers (experiment 4). A significant difference was observed in the number of colonies counted from the digesta obtained from the duodenum and this along with the strong tendency for different counts in the jejunum between the maize and commercial diet is worth noting. The fact that this difference was observed in adjoining sections where the counts were higher both in the duodenum and jejunum in birds from the maize diet group suggests that this may be a dietary effect. Because viscosity was not measured, it is difficult to explain why significant differences were not observed between maize and wheat-based diets but it has been noted by researchers in the past that the mean intestinal viscosity for maize can be higher than for wheat (Maisonnier *et al.*, 2001). The variability in pentosan content of different wheats may be a factor in the results obtained in this study.

The theory behind using a microbial organism is valid due to the potential effect reverse peristalsis might have on relocating bacterial populations. Future studies should employ the use of an inanimate marker that would adequately simulate bacteria, for instance fluorescent beads that will retain the same physical dimensions of a bacterium but will not be influenced by the gut environment.

Implications

The findings from this project hold significant implications for the poultry industry:

- The results demonstrate clearly the occurrence of reflux in both broiler and layer chickens. It is not exactly certain how this would affect productivity but further studies on the effects of diets would provide some insight into the implications.
- Digesta reflux appears to vary with the diet; implying that it may be possible to regulate the process and improve nutrient utilization.
- The use of markers in quantifying nutrient digestibility may need to be re-assessed. Although some investigation was conducted into the temporal nature of reflux, this cannot be related to a diurnal scale, to be able to determine what effect time of sample collection will have on measurements of digestibility.
- The results confirm the relocation of a microbial marker from posterior to anterior regions of the GIT. This has considerable implications for bird health, as organisms which are harmless in one region can be refluxed to other regions at which they can become pathogenic.

Recommendations

Most of the results of this study have been presented in the form of thesis at the University of New England. Some of the results were also presented at the recent Australian Poultry Science Symposium. There are plans to merge data from broiler and layer studies and prepare a manuscript for submission to a journal.

The student who worked on the project, Mr Adam Sacranie, has secured a scholarship to continue with his PhD at UNE. He will be applying for top-up scholarship from the CRC, to investigate the effects of different dietary factors on the incidence of digesta reflux. We believe that this will be a logical follow-up to the results presented in this report, and will greatly aid the application of this research.

Acknowledgements

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Plain English Compendium Summary

Project Title:	Reflux of digesta and its implications for nutrient digestion and bird health
Poultry CRC Project No.:	04-4 UNE
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Project Overview	Four experiments were conducted to evaluate the occurrence and possible effects of certain dietary ingredients on reverse movement of feed matter (reflux) in broiler and layer chickens. The first experiment, conducted on broiler chickens, was a preliminary trial, to investigate the occurrence of reflux in fasted and fed chickens. The results clearly indicated the prevalence of reflux throughout the small and large intestines of fed or fasted broiler chickens between 3 and 5 weeks of age. The second experiment looked at the effects of dietary fibre on the occurrence of reflux. The results confirmed the occurrence of reflux in fed and fasted chickens and the presence of oat hulls appeared to induce reflux. In the third experiment the passage of a bacterial marker and Cr-EDTA in broiler chickens fed diets based on maize or wheat was investigated. The results showed that both the bacterial marker and Cr-EDTA were relocated by reflux. No significant differences were observed between diet groups and marker levels in the various sections of the GIT. The fourth experiment was similar in design to the third experiment except that 39-week old layers were used. The Cr-EDTA marker was translocated towards the gizzard but such movement was less pronounced in chickens on diets based on maize. The antibiotic-resistant bacterium was refluxed to the upper gut. The results of the four experiments suggest that reflux appears to be a part of normal gut motility as well as a possible adaptive response to an absence of food. Dietary ingredients are very likely to affect reflux, especially ingredients that increase the viscosity of the digesta in the lumen. In addition, microbial populations may also be relocated by reflux, with serious implications for bird health.
Background	As with mammals, in birds, food matter progresses from the mouth to the cloaca (anus), during which useful nutrients are extracted. Some reverse movement has been observed in large birds, including the turkey, the implications of which unknown. The present study was conducted to examine if such reverse motion occurs in chickens and what this may imply for growth and health of chickens.
Research	
Project Progress	This study was completed in December 2006, and is in a position to submit a final report.
Implications	Our findings confirm the occurrence of reflux in chickens. The reverse movement of a potential disease-causing bacterium, in particular holds great implications for the poultry industry.
Publications	Two manuscripts have been presented at the Australian Poultry Science Symposium (2005 and 2007),