



Fructans from Renga Renga Lily (*Arthropodium cirratum*) Extract and Frutafit as Prebiotics for Broilers: Their Effects on Growth Performance and Nutrient Digestibility

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ABSTRACT : An experiment was conducted to evaluate the effect of dietary water-soluble carbohydrate extract from Renga renga lily (*Arthropodium cirratum*) and a commercial product, Frutafit® (both fructans) on the performance, organ weights, ileal digestibility and gut morphology of male Cobb broiler chickens. There were six treatment groups: a negative control with no supplements, a positive control supplemented with 45 ppm Zn-bacitracin, and four test diets each supplemented with Renga renga lily extract or Frutafit at 5 or 10 g/kg diet. Supplementation with low levels of Renga renga lily extract and Frutafit in the diet did not affect productive parameters, whereas the inclusion of a high level of Frutafit had a negative effect on BWG and FI compared with birds fed the negative control diet. The addition of an antibiotic to the diet significantly improved ($p < 0.05$) the BWG and FCR of broilers. Apparent ileal digestibility of dry matter, starch, protein and fat was not affected ($p > 0.05$) by supplementation with both levels of lily extract and the low level of Frutafit. The apparent ileal digestibility of dry matter, protein and fat was decreased ($p < 0.05$) by the high level of Frutafit. The apparent metabolisable energy (AME) of the diets fed the high level of Frutafit was approximately 0.2 MJ/kg DM lower than that of the negative control group. The addition of Zn-bacitracin increased ($p < 0.05$) the apparent ileal digestibility of fat. The relative weight of the liver was higher ($p < 0.05$) in broilers supplemented with the high level of Frutafit than for negative control birds at 14 and 35 d of age. Feeding Renga renga lily extract or Frutafit had no effect on the gut morphology of birds on d 14 and 35. It can be concluded that dietary inclusion of fructans from the two sources used in this study affected broiler performance differently and in a dose-dependent manner. (Key Words : Broiler Chickens, Digestibility, Fructans, Performance, Prebiotics)

INTRODUCTION

The use of in-feed antibiotics (IFA) in the poultry industry has been under scrutiny for the past decade due to growing concerns about the development of microbial resistance and the potential harmful effects on animal and human health. More recently, IFA were banned in the European Union. Around the world, there is a continuing search for suitable alternative(s) to IFA. Prebiotics are feed ingredients that can potentially affect the host's nutrition and health by selectively stimulating the activity of a number of beneficial gut microflora (Gibson and Roberfroid, 1995). Prebiotics may also affect mucosal morphology, probably because of their fermentation properties, which may strengthen mucosal protection and reduce the risk of

gastrointestinal diseases (Kleessen et al., 2003; Koutsos and Arias, 2006). However, information about how prebiotic and bioactive compounds affect gut morphology, digestibility of nutrients and performance of broilers is far from complete.

Potentially, there are hundreds of different prebiotics which are naturally available or can be produced from plant parts. Inulin and oligofructans are linear β -2 \rightarrow 1 fructans, widely distributed in nature as plant storage carbohydrates. Fructans are extracted primarily from chicory roots (*Cichorium intybus*) and Jerusalem artichoke (*Helianthus tuberosus*) tubers (Roberfroid, 2005). *Arthropodium cirratum* (Renga renga lily) is a small perennial herbaceous plant commonly available in Australia and New Zealand, which has starchy edible rhizomes rich in fructans as storage carbohydrate (Harris, 1996). Renga renga lily rhizomes contain about 65% fructans. The early settlers in Australia and New Zealand used the tubers of Renga renga lily as a food, as well as using them as a herbal medicine to treat boils and abscesses in humans (Harris, 1996; Cambie

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and Ferguson, 2003). The effects of fructans in Renga renga lily has not been studied in poultry.

The objective of the present study was to evaluate the effect of a water-soluble carbohydrate (WSC) extract from Renga renga lily and a commercially available prebiotic product, Frutafit[®], on growth performance, ileal digestibility, organ development, and gut morphology of broiler chickens.

MATERIALS AND METHODS

Bird husbandry

Two hundred and eighty-eight (288) day-old male broilers (Cobb), vaccinated against Marek's disease, infectious bronchitis, and Newcastle disease were obtained from a local hatchery (Baiada hatchery, Kootingal, NSW, Australia). At 1 d of age, chicks were randomly placed in 36 brooder cages with wire floor and with a floor space of 0.32 m²/cage. The cages were randomly assigned to one of six dietary treatments with six replicates per treatment. Each cage housed eight chicks. At 21 d of age, chickens from each replicate were placed into larger metabolic cages with a 1×1-cm wire mesh bottom and housed in climate-controlled rooms. The temperature was set at 33-34°C during the first week and gradually decreased by 3°C per week until 24-25°C was reached by the third week. Relative humidity was between 65 and 70%. A photoperiod of 24 h from 1 to 21 d of age, and 18 h from 22 to 42 d of age was maintained. Each pen was equipped with feed and water troughs latched to the outside of the cage and also an excreta collection tray. Water and feed were provided *ad libitum*. Weekly body weight gain and feed intake per cage were measured and feed conversion ratio (FCR), adjusted for mortality, calculated on a cage basis. Birds were observed twice daily for general health. The experiment was approved by the Animal Ethics Committee of the University of New England (Approval No.: AEC04/111).

Experimental diets

The composition of the basal diets is shown in Table 1. The diets were formulated to meet the requirements set by the National Research Council (1994) for broilers using the "PRO-4 Standard Edition" software package (Version 1, Agri-Data Systems, Inc., Annapolis, Maryland, USA). Celite[™] (Food Chemicals Codex grade, Celite Corp., Lompar, CA, USA), a source of acid-insoluble ash, was added (5 g/kg) to finisher diets as an indigestible marker. The grains in the experimental diets were hammer-milled using a 5-mm screen, and all diets were mixed and cold-pelleted at the University of New England. Treatments were as follows: i) basal diet with no additives (negative control); ii) basal diet with Zinc-bacitracin (45 mg/kg) (positive control); iii) basal diet with Renga renga lily rhizome

Table 1. Composition (g/kg as received) of experimental diets

Ingredient (g/kg)	Starter (1-21 d)	Finisher (22-35 d)
Sorghum	480	547
Corn	133	120
Soybean meal (48% CP)	274	230
Meat meal (50% CP)	80	56
Sunflower oil	7.5	10
Limestone (38% Ca)	5.0	5.8
Dicalcium phosphate	3.5	6.9
Lysine-HCl	1.2	1.7
DL-methionine	3.6	3.9
Salt	0.3	0.9
Sodium bicarbonate	5.0	5.0
Choline chloride	5.0	4.9
L-threonine	0.6	0.9
Celite	-	5.0
Premix ¹	2.0	2.0
Nutrient composition (g/kg)		
Metabolisable energy (MJ/kg)	12.3	13.1
Crude protein	230	205
Crude fat	39	46
Lysine	12	11
Methionine	7	7
Available phosphorous	5	4
Calcium	10	11

¹ Vitamin mineral premix (Ridley Agriproducts Pty Ltd., Tamworth, NSW) contained the following minerals in milligrams per kilogram of diet: Mn, 80; Zn, 60; Fe, 60; Cu, 8; I, 1.2; Co, 0.3; Se, 0.1; Mo, 1.0 and the following vitamins per kilogram of diet: Vitamin A, 12,000 IU from all *trans*-retinyl acetate; cholecalciferol D₃, 3,500 IU; vitamin E, 44.7 IU from DL- α -tocopherol; vitamin B₁₂, 12.75 μ g; riboflavin, 6.0 mg; niacin, 50 mg; pantothenic acid, 12 mg; folic acid, 2 mg; biotin, 0.1 mg; thiamine, 2 mg; vitamin K, 2 mg and pyridoxine, 5 mg.

extract (5 g/kg); iv) basal diet with Renga renga lily rhizome extract (10 g/kg); v) basal diet with Frutafit (5 g/kg) and vi) basal diet with Frutafit (10 g/kg). Additions of Frutafit[®]-HD, consisting of fructooligosaccharide (FOS) (Sensus, Roosendaal, The Netherlands), and Renga renga lily rhizome extracts consisting of 650 g fructans/kg were made in place of sorghum. The cleaned rhizome samples of Renga renga lily were air-dried, cut into small pieces and ground in a laboratory grinder (Mikro-Feinmuhle-Culatti MFC grinder, Janke and Kunkel GmbH and Co., Staufen, Germany). Powdered samples were extracted in boiling 80% (v/v) ethanol (1 g powder/50 ml ethanol) for 10 min and water-soluble carbohydrates were extracted twice with distilled water (50 ml/g, 70°C, 60 min). The ethanol and two water extracts were combined and concentrated *in*

vacuo at 40°C in a rotary evaporator (Buchi Rotavapor-R, Buchi Laboratories, Flawil, Switzerland). After freezing and thawing, the resulting insoluble materials were removed by centrifugation at 3,500×g for 20 min at 20°C using an MSE Mistral 2000R centrifuge (Sanyo Gallenkamp PLC, Leicestershire, UK), and the supernatants were passed through an ion exchange column consisting of both anion exchange resin (Amberlite IR 120, Na⁺ form) and cation exchange resin (Amberlite IR 401, Cl⁻ form). The neutral elutes were immediately frozen in acetone cooled with dry-ice, and lyophilized for 72 h at -49°C and 62×10⁻³ mbar (Eyela Freeze Dryer FD-1, Rikakikai Co., Tokyo, Japan). The freeze-dried powder of the extract (off-white) was incorporated into experimental diets.

Frutafit[®]-HD contains mainly inulin (920 g/kg) (fructooligosaccharides) extracted from chicory root and some fructose and glucose (40 g/kg) and sucrose (40 g/kg). The average chain length of the inulin in Frutafit is about 9-11 carbon atoms and the content of fructooligosaccharides (with DP below 10) is about 25-30% with about 2-3% DP3 and DP4, and 3-4% of DP5.

Collection and processing of samples

Birds were killed by cervical dislocation on d 14 (one bird per replicate) and 35 (three birds per replicate). On d 35, three chickens were selected at random from each replicate and euthanized. To synchronise the feeding pattern of the birds, light was switched off for 2 h, followed by at least 1 h light before the chickens were sacrificed. Subsequently, the abdominal cavity was opened and the small intestine was ligated and removed. The contents of the ileum were collected into plastic containers. The ileal digesta samples were frozen immediately after collection, subsequently lyophilized (Martin Christ Gelfriertrocknungsanlagen, GmbH, Osterode am Harz, Germany), ground to pass through a 0.5 mm sieve (Cyclotec 1093 sample mill, Tecator, Höganäs, Sweden), and stored at -20°C in airtight containers until chemical analyses were conducted. On d 14 and 35, approximately 2.5 cm of the middle portion of the ileum was excised from one bird per replicate, flushed with PBS buffer (pH 7.6) and fixed in 10% (v/v) neutral buffered formalin for histomorphological analysis.

Organ weights

The weights of the proventriculus, gizzard, and small intestine without content, pancreas, bursa of Fabricius, caeca, spleen and liver without gall bladder were recorded on d 14 and d 35. The duodenum is the region from the outlet of the gizzard to the distal attachment of the pancreas, the jejunum, distally from the end of the pancreatic loop to Meckel's diverticulum, the ileum distally from Meckel's diverticulum to 1 cm above the ileo-caecal junction. The

weight of each segment was recorded, as was the body weight of the bird they were excised from.

Apparent metabolisable energy (AME) bioassay

Apparent metabolisable energy (AME) evaluation was conducted over a period of 4 days during the fifth week of the experiment (36 to 39 d post-hatch). Clean excreta trays were placed under each cage, droppings were collected daily, dried at 80°C to a constant weight in a forced-drought oven and collections from each pen were pooled for analysis. Care was taken to avoid contamination with feed, feathers, scales and debris. The moisture content of the excreta voided was measured. Diet and excreta were ground to pass through a 0.5 mm screen using a Cyclotec sample mill. Gross energy contents of diets and excreta were determined using an IKA bomb calorimeter system, C7000 with Cooler C7002 (IKA[®]-Werke GmbH and Co, Staufen, Germany) standardized with benzoic acid. Apparent metabolisable energy of diets was calculated using the equation below and values were corrected for zero nitrogen retention using a value of 34.4 MJ per g nitrogen retained (Hill and Anderson, 1958).

$$\begin{aligned} \text{AME diet (MJ/kg DM)} \\ = \text{GE}_{\text{diet}} - (\text{GE}_{\text{excreta}} \times (\text{AIA}_{\text{diet}} / \text{AIA}_{\text{excreta}})) \end{aligned}$$

where, GE_{diet} = gross energy content in diets

$\text{GE}_{\text{excreta}}$ = gross energy content in excreta

AIA_{diet} = Acid-insoluble ash content in diets

$\text{AIA}_{\text{excreta}}$ = Acid-insoluble ash content in excreta or ileal digesta.

Digestibility of nutrients

Apparent ileal digestibilities of protein, fat, starch and DM and the AME as a proportion of the gross energy of feed were estimated from the analyses of feeds, freeze-dried ileal digesta and excreta. Diets and ileal digesta were analysed for DM, protein, fat and starch as described below. The apparent ileal digestibility of protein, fat, starch and DM were calculated using the following formula. All values are expressed on a DM basis.

$$\begin{aligned} \text{Apparent nutrient digestibility} \\ = \frac{(\text{Nutrient/AIA})_{\text{diet}} - (\text{Nutrient/AIA})_{\text{ileum}}}{(\text{Nutrient/AIA})_{\text{diet}}} \end{aligned}$$

where, $(\text{Nutrient/AIA})_{\text{diet}}$ = ratio of nutrient and acid insoluble ash in diet, and

$(\text{Nutrient/AIA})_{\text{ileum}}$ = ratio of nutrient and acid insoluble ash in ileal digesta.

Acid-insoluble ash

The concentration of AIA in the feed, freeze-dried ileal

digesta and excreta was determined after ashing the samples and treating the ash with boiling 4 M HCl, following the method described by Vogtmann et al. (1975) and Choct and Annison (1990). Samples (diet, 3 g; ileal digesta, 1 g) were weighed accurately into Pyrex® brand Gooch-type crucibles (porosity 4 µm) and dried (overnight, 105°C) in a forced-air convection oven (Qualtex Universal Series 2000, Watson Victor Ltd., Perth, Australia). After cooling and weighing, the samples were ashed (480°C, overnight) in a Carbolite CWF 1200 chamber furnace (Carbolite, Sheffield, UK). The crucibles were placed in a boiling 4 M HCl acid bath so that the samples were wetted from underneath (just covered the ash). The samples were gently boiled twice in 4 M HCl for 15 min and the acid was removed through suction, the residues (AIA) were washed with distilled water, and the crucibles were dried (105°C, overnight) and weighed. The AIA content was calculated using the following equation:

$$\text{AIA (g/kg dry matter)} = \frac{(\text{Crucible + ash weight}) - (\text{Crucible weight})}{(\text{Crucible + dry sample weight}) - (\text{Crucible weight})} \times 1,000$$

Nutrient analyses

The total starch content of the diets and ileal digesta was determined using the Megazyme Total Starch Assay Kit (Megazyme Australia Pty. Ltd., Warriewood, NSW, Australia) based on the method developed by McCleary et al. (1994). When calculating starch digestibility it was assumed that the free glucose in ileal digesta derives from starch.

Diets and ileal digesta were analysed for protein by the method of Sweeney (1989) using a LECO® FP-2000 automatic nitrogen analyser (Leco Corp., St. Joseph, MI, USA). The crude fat content of the ileal digesta samples was determined gravimetrically by the Soxhlet extraction procedure using the Association of the Official Analytical

Chemists Official Method 920.39 (AOAC, 2002).

Statistical analysis

Each variable was analysed as a completely randomized design with a cage of broilers composing an experimental unit. Percentage data were arcsine-transformed prior to analysis and data from all the response variables were analysed according to the General Linear Models procedure (GLM) for ANOVA (SAS Institute Inc., 2000). Variables having a significant *F* test were compared using Duncan's Multiple Range Test. Differences at $p \leq 0.05$ were considered significant, unless otherwise stated.

RESULTS

Bird performance

The growth performance of broilers is shown in Table 2. Mean BWG of the group on Renga renga lily extract (10 g/kg) during the first three-week period was similar to that of the negative control group and that of birds fed the antibiotic during the same period. Mean BWG in the group supplemented with high level of Frutafit was lower ($p < 0.05$) than that of the negative control group throughout the experimental period. The overall FI (1 to 42 d) and FI during the first three weeks (1 to 21 d) were lower ($p < 0.05$) in the same treatment group. Feed intake of all other treatment groups was not different from the negative control group. Feed efficiency was not affected by dietary supplementation with lily extract or Frutafit. Addition of 45 mg/kg Zn-bacitracin resulted in significant ($p < 0.05$) improvements in BWG and FCR of birds compared to those fed the negative control.

Visceral organ weights

The relative weights of major visceral organs and regions of the intestine are shown in Table 3. The relative

Table 2. Mean body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of broiler chickens fed diets supplemented with the Renga renga lily extract and Frutafit¹

	Period	Negative control	Positive control	Renga renga lily extract		Frutafit		SEM
				5 g/kg	10 g/kg	5 g/kg	10 g/kg	
BWG (g/bird)	1-21 d	823 ^b	853 ^a	832 ^b	838 ^{ab}	828 ^b	781 ^c	17.6***
	22-42 d	1,851 ^b	1,926 ^a	1,852 ^b	1,858 ^b	1,853 ^b	1,831 ^c	15.7***
	1-42 d	2,668 ^b	2,779 ^a	2,678 ^b	2,681 ^b	2,664 ^b	2,613 ^c	23.5***
FI (g/bird)	1-21 d	1,392 ^a	1,407 ^a	1,399 ^a	1,407 ^a	1,405 ^a	1,336 ^b	15.2***
	22-42 d	3,716	3,722	3,721	3,716	3,713	3,680	39.8
	1-42 d	5,116 ^a	5,129 ^a	5,120 ^a	5,121 ^a	5,096 ^a	5,048 ^b	45.5*
FCR	1-21 d	1.69 ^{ab}	1.65 ^b	1.68 ^{ab}	1.68 ^{ab}	1.70 ^a	1.71 ^a	0.04*
	22-42 d	2.01 ^a	1.93 ^b	2.01 ^a	2.00 ^a	2.00 ^a	2.01 ^a	0.03***
	1-42 d	1.92 ^a	1.85 ^b	1.91 ^a	1.91 ^a	1.91 ^a	1.93 ^a	0.02***

¹ Least square means and pooled standard error of the mean (SEM), $n = 6$.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, results not sharing the same superscripts within a row are significantly different ($p < 0.05$).

Table 3. Effect of Renga renga lily extract and Frutafit on relative weight of organs (g/100 g body weight) of broilers at 14 and 35 days of age¹

	Liver		Pancreas		Caeca		Duodenum		Jejunum		Ileum	
	14 d	35 d	14 d	35 d	14 d	35 d	14 d	35 d	14 d	35 d	14 d	35 d
Negative control	3.90 ^b	2.56 ^b	0.44 ^{ab}	0.19	0.81	0.50 ^b	1.72	0.71	2.62 ^a	1.13 ^a	1.58	0.90
Positive control	3.92 ^b	2.48 ^b	0.38 ^b	0.20	0.78	0.48 ^b	1.77	0.69	2.08 ^b	1.00 ^b	1.51	0.79
Lily extract (5 g/kg)	4.10 ^b	2.63 ^{ab}	0.42 ^{ab}	0.21	0.64	0.51 ^b	1.95	0.70	2.68 ^a	1.12 ^a	1.61	0.87
Lily extract (10 g/kg)	4.07 ^b	2.51 ^b	0.43 ^{ab}	0.22	1.06	0.61 ^{ab}	1.56	0.69	2.50 ^{ab}	1.11 ^{ab}	1.88	0.87
Frutafit (5 g/kg)	3.84 ^b	2.66 ^{ab}	0.40 ^{ab}	0.21	0.67	0.56 ^{ab}	1.82	0.72	2.59 ^a	1.15 ^a	1.55	0.91
Frutafit (10 g/kg)	4.77 ^a	2.95 ^a	0.52 ^a	0.20	0.81	0.66 ^a	2.03	0.78	2.94 ^a	1.20 ^a	1.88	0.95
SEM	0.53 ^{**}	0.31 [*]	0.11 [*]	0.05	0.41	0.12 [*]	0.50	0.10	0.43 ^{**}	0.11 [*]	0.44	0.15

¹ Least square means and pooled standard error of the mean (SEM), $n = 6$.

^{a,b} Mean values not sharing the same superscripts within a column are significantly different at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

weight of the liver was highest in birds on the high Frutafit diet at d 14 ($p < 0.01$) and d 35 ($p < 0.05$). In the same treatment group the relative weight of the pancreas was greater ($p < 0.05$) than that of positive control, but not significantly different from the negative control at d 14. The relative weight of the caeca was greater ($p < 0.05$) in birds fed the high level of Frutafit compared to both negative and positive control groups at d 35. The relative weight of the jejunum of birds fed with Zn-bacitracin-supplemented diet was lower than that of birds on the other diets. Dietary supplementation with Renga renga lily extract or Frutafit had no effect on the relative weights of the proventriculus, gizzard or immune organs (bursa and spleen) on d 14 and 35 (data not shown).

There was no effect ($p > 0.05$) of any treatment on the mucosal morphometry of the jejunum although Zn-bacitracin tended to decrease ($p < 0.10$) the crypt depth on d 35 (data not shown).

Ileal digestibility of nutrients

The apparent ileal digestibility of nutrients is shown in Table 4. Renga renga lily extract supplementation at both levels had no effect on apparent ileal digestibility of nutrients. The apparent ileal digestibility of dry matter, protein and fat was decreased ($p < 0.05$) in birds fed Frutafit at the high level. The apparent ileal digestibility of fat was

higher ($p < 0.05$) in the antibiotic fed group compared with the other treatment groups. High level of Frutafit decreased ($p < 0.05$) AME of birds between d 36 to 39, while the other dietary treatments showed no difference in AME compared to the negative control group (Figure 1).

DISCUSSION

Dietary supplementation with a high level of Renga renga lily extract did not affect BWG compared to the negative control group and produced BWG equal to that of birds fed on antibiotic-containing diet during the first 3-week period. This is in contrast to the results by Yusrizal and Chen (2003) who observed a significant improvement in BWG and FCR in female broilers when they were fed diets supplemented with chicory fructans (10 g/kg). In the current study, the high level of Frutafit, but not the low level, significantly depressed the weight gain of the birds. Similar results have been shown in chickens fed diets supplemented with high levels of fructans (Wu et al., 1999; Chen et al., 2005). The reduction in BWG in the group supplemented with the higher dosage of Frutafit was due primarily to a reduction in FI. Possibly, the digestion of large amounts of fructans is hindered in these birds, leading to an accumulation of indigestible material in the intestine, which then is degraded by microbes resulting in flatulence.

Table 4. Apparent ileal digestibility (%) of major nutrients in broilers fed the experimental diets¹

	Dry matter	Starch	Protein	Fat
Negative control	72.59 ^a	93.57	77.21 ^a	74.32 ^b
Positive control	72.62 ^a	93.81	77.19 ^a	75.29 ^a
Lily extract (5 g/kg)	72.45 ^a	93.71	77.27 ^a	74.34 ^b
Lily extract (10 g/kg)	72.44 ^a	93.73	77.26 ^a	74.32 ^b
Frutafit (5 g/kg)	72.56 ^a	93.71	77.27 ^a	74.50 ^b
Frutafit (10 g/kg)	71.02 ^b	93.48	76.04 ^b	70.76 ^c
SEM	0.73 ^{***}	0.32	0.60 ^{**}	0.73 ^{***}

¹ Least square means and pooled standard error of the mean (SEM), $n = 6$.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, results not sharing the same superscripts within a column are significantly different ($p < 0.05$).

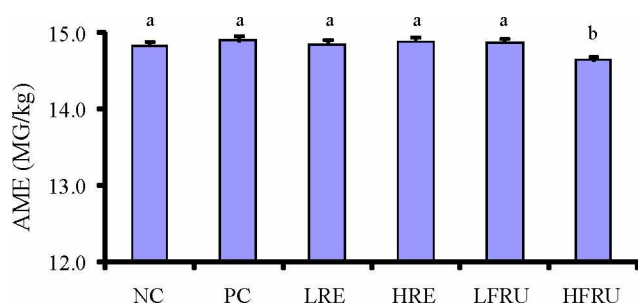


Figure 1. Effects of Rengga lily extract and Frutafit on AME of diets between 36 to 39 days of age. Negative control (NC), positive control (PC), low level of Rengga lily extract (LRE), high level of Rengga lily extract (HRE), low level of Frutafit (LFRU) and high level of Frutafit (HFRU). (Mean values, $n = 6$; error bars indicate pooled SEM; bars with different superscripts are significantly different).

a feeling of satiety, and thus reduction in FI (Iji and Tivey, 1998). As observed in the present study, Waldroup et al. (1993) reported that the inclusion of a low level of fructans (4 g/kg) in broiler diets did not have any positive effect on production parameters. The findings of this study agree with many observations that dietary Zn-bacitracin significantly improves the performance of broilers (Stutz and Lawton, 1984; Stanley et al., 2004; Ravindran et al., 2006).

The relative weights of the major digestive as well as immune organs were not affected by supplementation with Rengga lily extract or the lower level of Frutafit. The significant enlargement of the liver and pancreas of birds fed on the higher level of Frutafit may be attributed, partly, to the presence of a high concentration of indigestible oligosaccharides in the GIT. Perhaps the large amount of oligosaccharides in Frutafit may have acted as an anti-nutritional factor, inducing hypertrophy and hyperplasia in these organs. An accumulation of undigested oligosaccharides may cause an increase in the relative weight of the caeca in the high Frutafit-fed group. In line with this, Jozefiak et al. (2004) reported that high dietary fibre components affected fermentation in poultry caeca, leading to caecal hypertrophy. A significant decrease in the relative weight of jejunum and numerically shorter relative lengths of jejunum and ileum following antibiotic supplementation are presumably related to a reduction in microbial populations and more efficient utilisation of nutrients. A similar effect of antibiotics on intestinal weight of broilers has been confirmed by earlier investigators (Stutz and Lawton, 1984; Dafwang et al., 1985; Sarica et al., 2005). By reducing populations of pathogenic bacteria in the intestine, antibiotics prevent the accumulation of lymphocytes in the epithelial layer and the underlying lamina propria, which, in turn results in thinning of the

muscularis in intestine (Coates, 1980; Gunal et al., 2006). Reduction in intestinal weight due to thinning of the intestinal mucosa may also enhance nutrient absorption by the host (Visek, 1978; Henry et al., 1987). The improved fat digestion in birds fed with Zn-bacitracin in the current study also supports this hypothesis.

The apparent ileal digestibility of dry matter, fat, protein and AME was lowered in broilers fed with the high level of Frutafit. A high concentration of fructans in the GIT of broilers may lead to an increase in microbial fermentation in the lower GIT that could adversely affect the utilization and absorption of nutrients, such as protein and fat. Due to the lack of the enzyme galactosidase in the small intestine of birds, it can be assumed that break down of fructans in the distal intestine occurs by bacterial fermentation. Chen et al. (2005) have observed a reduction in pancreatic lipase in chickens fed with a high level (10 g/kg) of fructans and suggested that it could lead to lower lipid digestibility. Similar to the findings in the present study, Leske et al. (1993) found that adding high levels of oligosaccharides to poultry diets reduced the true metabolisable energy in a dose-dependent manner. Furthermore, Coon et al. (1990) suggested that rapid intestinal transit of digesta caused by an increase in acidity resulting from the microbial fermentation of oligosaccharides, could reduce the metabolisable energy of diets in chickens.

The reduction in the ileal digestibility of protein in birds that were fed the high level of Frutafit indicates that more protein was recovered in the ileal digesta, which in turn may be partly due to an increase in bacterial cell synthesis. Ravindran et al. (1999) suggested that the fermentation of carbohydrate components in the hindgut is likely to be responsible for the net microbial protein synthesis in broilers. Although not assessed, the high concentration of fructans may increase the rate of passage of digesta in the upper small intestine; this may prevent proteolytic enzymes from acting fully on dietary proteins. Therefore, the reduction in performance of birds fed the high level of Frutafit may also be explained by the decrease in ileal nutrient digestibility, and AME, apart from the low FI observed with the same group.

The reason that the high level of Rengga lily extract had no effect on ileal digestibility of nutrients in this study may be because the concentration of fructans in lily extract is low (650 vs. 920 g/kg) compared to Frutafit. The low concentrations of fructans may slow down the microbial enzyme reactions because of the limitation of substrate. The other possibility is that the difference in average DP in fructans from two different sources can elicit varying responses. The average DP of fructans present in Frutafit is 9-11, whereas in Rengga lily extract it is >12. By measuring fructans disappearance, Roberfroid et al.

(1998) indicated that the long-chain fructans fermented at least twice as slowly as their short-chain counterparts.

CONCLUSION

High levels of fructans in broiler diets may have no effect or negative effect on growth and nutrient utilisation. At low levels, both the commercial and natural supplements tended to alter nutrient utilization and gastrointestinal physiology but this did not translate into an improvement in gross response. It is not certain what the response to these supplements would be if birds are raised on litter, with access to their droppings and possibly changes in microbial profiles.

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