Digestibility of Nutrients on Broiler Given Various Protein Sources and Two Levels of Quebracho Tannin

(Kecernaan Nutients pada Broiler yang Mendapat Berbagai Sumber Protein dan Dua Taraf Quebracho Tannin)

Rusdi

Feed and Nutrition Section Faculty of Agriculture, Tadulako University

Abstrak

Suatu penelitian telah dilaksanakan yang bertujuan untuk melihat pengaruh sumber protein dan kandungan tannin dalam ransum terhadap kecernaan nutrien pada ayam broiler. Sebanyak enampuluh empat ayam telah digunakan dan ditempatkan secara acak dalam faktorial 2x4 sebanyak delapan ulangan. Protein yang digunakan meliputi gelatin, tepung ikan, tepung bulu dan campuran tepung daging dan kedelai. Hasil penelitian menunjukkan bahwa Quebracho Tanin (QT) menekan kecernaan nutrien pada broiler. Sumber protein mempunyai kemampuan untuk mengoreksi pengaruh negatif tanin dimana tepung ikan menghasilkan nilai tertinggi dan tepung bulu menghasilkan nilai yang paling rendah pada kecernaan. Nilai kecernaan protein masing-masing untuk tepung ikan dan tepung bulu sebesar 54,40 dan 44,30%.

Kata Kunci : Kecernaan, quebracho, tannin

Introduction

Tannins generally reduce feed palatibility, voluntary intake, protein and carbohydrate digestibility (Mangan, 1988) and thereby reduce the growth rate. Some animals such as deer and rats have ability to anticipate the negative effects of tannins by producing proline rich protein (PRP) from their saliva that binds tannin and voids it in the faeces. Animals such as cattle and chicken do not produce PRP and in fact they cannot entirely survive to the tannin containing diet.

Particular protein has ability to bind tannin where gelatine bind more tannin than bovine serum albumin (BSA) does (Rusdi, 2004). This is due to gelatine high in proline content that forms an imide instead of amide bond with tannins, which cannot be hydrolysed by endogenous enzymes in mammals. Thus, gelatine may improve digestibility of nutrients in livestock given a tannin containing diet, and others commonly protein meals might also have ability to eliminate the negative effects of tannins as protein meal varies in their proline content and nature. The objective of the following study was assessing the ability of particular proteins to cope with a deteriorating effect of tannin in the digestibility.

Research and Methods

This research was carried out at the poultry Researchand Development Centre of Department of Primary Industry of Queensland. The site is located at Alexander Hills 30 km east of Brisbane.

Preparation of Tannin – Protein Mixtures and Diets

Diets were isonitrogenously and isoenergeticly formulated according to the Recommended Nutrient Requirement of Chickens (NRC, 1994). The basal diet consisted of mixture of corn, wheat, soybean, meatbone meal, oil, minerals and vitamins. Gelatine, fish meal, feather

meal or soy-meat meal (a mixture of 50% of soya bean meal and 50% of meat bone meal on weight basis) as additional protein sources were variously added to the basal diet to each generate an additional 10% crude protein (CP) im the final diet. Gelatine was limited to only 2.50% of the total diet. Quebracho tannin extract (QT) containing 73.40% of CT, was added at the level 5% of the final diet on a dry matter basis as +QT diet and non QT as - QT. Chromium dioxide (Cr_2O_3) was added (0.15%) to all diets as marker. The composition of these diets is shown in Table 1.

A complexation of tannin-protein were prepared by mixing QT and particular proteins in a bucket with phosphate buffer (50mM, pH 6.50, containing 0.10% ascorbic acid). Mixtures were kept at room temperature for 2 h to alow the formaion of the protein tannin complex and then dried in a dehydrator for 24 h at 45-50^{O}C. All ingredients were thoroughly mixed in the automatic mixer. The final mixture was divided into 8 equal parts, one for each treatment. The control diets from each particular protein were prepared by replacing QT with a-5% of rice hull. All diets were then pelleted in th cooled press machine.

Housing and Design of the Experiment

One day old male-broiler chickens, strain Cobb, were bought from a commercial hatchery at Darewalla Farm. They were reared in a group on a commercial starter mash (Riverina, Queensland) for up to two weeks, under the same conditions of space, light, temperature and humidity for each lot. The temperature was set at 32^{O}C for one day of age and reduced by approximately a 1^{O}C each day. At 15 days old, chickens were weighed individually and sixty-four chickens of middle weight range (392±30g) from two lots were selected and randomly allocated to the individual battery cages and were kept in these cages for 28 days. Each cage was equipped with an individual feeder, automatic drinker, and a tray for excreta collection. The caged bird were placed on the multilevel deck shelf in the semi-controlled shed. The temperature was 16-22^{O}C and the artificial lighting period was 23 h/d. Feed and drinking water were freely available. On day fifteen, slected chickens were allocated randomly to a factorial design with four protein source and two levels of tannin within eight replicates. The first factor was protein source of either gelatine, feather meal, fish meal or soya-meat meal. The second factor was two levels of QT (0 or 5%).

Measurement and Sampling

Intake of chickens was individually measured by subtracting feed offered and feed refusals on a daily basis. Feed offered and feed refusals were sampled for later analysis.

The digestibility of dry matter, organic matter and nitrogen of excreta was conducted using an internal marker method in which chromic dioxide (Cr_2O_3) was used as an indigestible marker. At 35 days, clean excreta trays were placed under each battery cage for 7 day period. A clean sample of excreta was collected in a 250 mL specimen jar every two hours/d and immediately stored at – 20^{0}C. Excreta samples were well mixed and freeze-dried and ground and stored at – 5^{O}C for later analysis.

The digestibility of N was calculated as described by Ravindran *et al*. (1999) using the following equation : Digestibility of N (%) = 100{(N/Cr in diet-N/Cr in excreta)/N/Cr in diet)}, where N and Cr are the concentration of nitrogen and chromium respectively in either diet or excreta.

Table 1. Composition and calculated analysis of experimental diets (%) on dry matter basis with or without Quebracho Tannin

	Diet							
	Gelatine		Feather meal		Fish meal		Soy-meat meal	
	+QT	- QT	+QT	- QT	+QT	- QT	+QT	- QT
Ingredients (%)								
Maize	43	43	43	43	43	43	43	43
Wheat	19.50	19.50	19.50	19.50	19.50	19.50	19.50	19.50
Soybean	5	5	5	5	5	5	5	5
Meat	5	5	5	5	5	5	5	5
Premix*	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Oil	1.70	1.70	4	4	1.80	1.80	0.80	0.80
Ca-carbonat	0	0	2	2	0.25	0.25	0	0
Ca-phosphate	0.10	0.10	1.80	1.80	0	0	0	0
DL-Methionine	0.35	0.35	0.65	0.65	0.25	0.25	0.30	0.30
L-Lysine hydrochloride	0.20	0.20	0.65	0.65	0.20	0.20	0.20	0.20
L-Threonine	0.10	0.10	0	0	0	0	0.05	0.05
Cr_2O_3	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Gelatine	2.50	2.50	0	0	0	0	0	0
Feather meal	0	0	12.40	12.40	0	0	0	0
Fish meal	0	0	0	0	19.45	19.45	0	0
Soya-meat meal	17	17	0	0	0	0	20.60	20.60
Rice hull	0	5	0.45	0.45	0	5	0	5
Tannins	5	0	5	0	5	0	5	0
Composition								
Crude protein, %	19.13	1913	18.77	18.77	18.65	18.65	18.60	18.60
ME, MJ/kg	10.88	10.88	11.22	11.22	11.33	11.33	10.62	10.62
Ca, %	1.41	1.41	1.61	1.61	1.55	1.55	1.56	1.56
P, %	0.87	0.87	0.86	0.86	0.94	0.94	0.94	0.94
Methionine, %	0.62	0.62	0.86	0.86	0.7	0.7	0.58	0.58
Lysine, %	1.09	1.09	1.18	1.18	1.39	1.39	1.10	1.10
Cysteine, %	0.28	0.28	0.67	0.67	0.29	0.29	0.30	0.30
Threonine, %	0.73	0.73	0.77	0.77	0.84	0.84	0.71	0.71
Thriptophan, %	0.17	0.17	0.15	0.15	0.23	0.23	0.19	0.19

*) The vitamin and mineral premixes added per kg of diet : 8332.5 iu retinol, 3000 iu cholecalciferol, 23.766 mg Di-alpha-tocopheryl acecate, 2 mg hetrazeen, 1 mg thiamine, 4 mg riboflavin, 2 mg pyridoxine HCl, 0.01 mg cyanocobalamin, 43.33 mg niacin, 10 mg Ca pantothenate, 1 mg folic acid, 0.172 mg biotin, 88 mg Banox, 0.5 mg Co, 4 mg Cu, 50 mg Fe, 0.6 mg I, 0.07 mg Se, 0.6 mg Mo, 50 mg Mn, 50 mg Zn.

Analytical and Statistical Procedures

Diets and excreta samples were analyzed for dry matter (DM), organic matter (OM), nitrogen (N) and Cr_2O_3. DM was estimated as the residue remaining after samples were dried at 65^OC for 48 h. Sample were ashed at 550^OC for 5 h, the loss in weight representing the OM content. The N content of the samples was determined using a Leco CNS-2000 Combustion Analyzer (Leco Corporation, USA). Amino acid profiles of the diets were analyzed at the Amino Acid Laboratory of ARI of Queensland Primary Industry Department. All values are expressed on dry matter basis.

For the determination of chromium, samples of diet and excreta were cold digested overnight with 15 mL of a mixture of concentrated perchloric acid and concentrated nitric acid (1:5 respectively). The digestion flasks were then transferred to a digestion block and heated slowly,

boiling off nitric acid. The temperature was progressively raised until perchloric acid started to evolve as white fumes and the digests colour became yellow. The digests were allowed to cool, transferred to a 25 mL volumetric flask and made up to mark. These concentrations were measured against a low and high chromium standard on an Inductively Coupled Plasma Atomic Emission Spectrometer (ICP) (Spectro, Spectroflame P, 1993). Tannins analysis is formed in Rusdi (2004).

Data were analysed using an analysis of variance as a factorial design (Steel and Torrie, 1980) by means of General Linear Model of SAS (SAS, 1998). The factors were protein source (4) and QT level (2) within 8 replicates. Treatment means were tested for significant treatment effects by LSD analysis.

Result and Desaign

Diet Composition and Tannin Content

The composition of the diets in presented in Table 2.

Effects of tannin

The chickens fed the crude extract of QT exhibited significantly ($P<0.05$) lower in dry matter digestibility, organic matter digestibility compared with the chickens fed the tannin-free diets (Table 3). While dry matter intakes of chickens on the CT diets was lower ($P>0.05$) than that of chickens in the tannin-free group.

Table 2. Dry matter (DM), organic matter (OM), nitrogen (N) and condensed tannin(CT) content of experimental diets on a dry matter basis

	Diet							
	Gelatine		Father meal		Fish meal		Soy-meat meal	
	+ QT	- QT	+ QT	- QT	+ QT	- QT	+ QT	- QT
DM (g/kg)	916.80	915.40	915.20	918.40	919.10	918.20	914.30	913.10
OM (g/kg)	929.10	917.00	922.40	917.00	915.3	903.40	920.20	914.00
N (g/kg)	38.00	38.00	38.00	38.00	37.0	37.00	37.00	38.00
Amino acids (%)								
Arginine	1.46	1.48	1.67	1.57	1.41	1.30	1.51	1.36
Histidine	0.51	0.49	0.51	0.40	0.61	0.54	0.53	0.50
Lysine	1.08	1.17	0.94	0.68	1.23	1.29	1.15	1.14
Methionine	0.45	0.48	0.67	0.64	0.64	0.55	0.49	0.44
Proline	1.75	1.75	1.95	2.02	1.40	1.43	1.65	1.55
CT (g/kg)								
Free	53.80	ND	54.10	ND	52.70	ND	51.10	ND
Protein bound	2.80	ND	2.10	ND	3.60	ND	2.60	ND
Fibre bound	0.60	ND	0.60	ND	1.60	ND	0.50	ND
Total	57.10	ND	56.80	ND	57.90	ND	54.20	ND

ND not detected

Table 3. Effect of tannin (QT) on dry matter intake (DMI), digestibility of dry matter (DMD), organic matter (OMD), nitrogen (ND) of chickens fed tanin containing or tannin free diet with one of either gelatine, feather meal, fish meal or soya-meat meal included for a 28 days feeding period.

Level of QT (%)	DMI (g/day)	DMD (%)	OMD (%)	ND (%)
0	101.80	70.12	74.82	56.89
5	101.00	61.09	65.21	39.96
SEM#	1.70	0.01	0.01	0.01
P*	0.7219	0.0001	0.0001	0.0001

standard error mean; * probability

Effects of protein

There were significant (P<0.0002) effects of protein sources on dry matter intake (P<0.03) and nitrogen digestibility (P<0.0003), but neither dry matter nor organic matter intake digestibility was significantly (P>0.05) affected. Fish meal induced higher dry matter intakes when compared with gelatine (P<0.01) and soya-meat meal (<0.007). Organic matter digestibility in chickens fed fish meal resulted in a higher than for those fed feather meal (P<0.03) and gelatine (P<0.05). Similarly, fish meal resulted in a higher nitrogen digestibility compared with feather meal and soya-meat (P<0.006) and gelatine (P<0.02).

Interaction of Protein Sources and Tannin Levels

QT significantly decreased dry matter intake in all protein types except gelatine. Gelatine, on the other hand, resulted in increased dry matter intake. QT consistently depressed digestibility of nutrients within protein sources (P<0.001), except QT in the feather meal diet which did not significantly decrease the digestibility of dry matter and organic matter (P>0.05), and significantly decreased the digestibility of nitrogen (p<0.05).

It has been proposed that the major anti-nutritional effect of tannin is to inhibit dietary protein digestion (Jansman, 1993). Thus, high concentrations of protein in the faecces are possibily due to tannins binding to endogenous and/or exogenous protein sources (Mitjavila *et al.,* 1977; 1989), and or a part of mucous and intestinal sell (Mitjavila *et al.,* 1977). However, the present study could not distinguish which proteins in the excreta were generated from endogenous or exogenous sources, and it was assumed that the indigestible proteins in the faeces were derived from both sources.

A severe reduction in the digestibility of drymatter, organic matter and nitrogen in the present study accords well with the previous findings of Mahmood and Smithard (1993) andthe results of lambs and Acamovic (1998). CT has been reported to bind not only protein (endogenous enzymes and amino acids) but also dietary carbohydrate or minerals (Mangan, 1988). Silanikove *et al*. (1994) reported that CT may depress the intestinal activity of trypsin and amylase, and that these tannin-bound proteins would probably be resistant to protease action (Van Soest, 1994). Furthermore, Dawson *et al*. (1999) reported that QT erodes the inner lining of the small intestine, particularly the jejunum and ileum, suggesting QT may be locally toxic to the surface epithelium in this region of the digestive tract, which in turn may reduce the absorption of nutrients. Consequently, the apparent digestibility of nutrients in chickens fed Qt was everely

depressed. Thus, the adverse biological effects resulting from the consumption of QT by chickens is most likely due to a combination of condensed tannin-associated impairment of protein utilization (both dietary and endogenous) and reduction in the ability of the small intestine to absorb nutrient due to tannin bound protein reducing availability and the activity of digestive enzymes and the impairment of the functional activity of the intestine.

Chickens fed fish meal had better dry matter intake and digestibility of nutrients as compared to those fed the other protein sources. Chickens fed gelatine showed similiar trends, in terms of intake and digestibility, to chickens fed soya-meat diets. The inclusion of feather meal seems to have lowered the digestibility of nutrients and to be associated with poor growth rates. Gelatine and soya-meat meal diets gave similiar responses in chicken growth and performance, mainly because soya bean and meat meal proteins represented the major proteins in both rations (Table 1). However, the performance of chickens given these diets was markedly lower than that of chickens given fish meal. Both gelatine and soya-meat meal diets were poorly digested by chickens in the present trial, and their poor performance may have been further aggravated by the presence of toxic and/or inhibitory substances such as protease inhibitors in these meals (Mc Donald *et al.,* 1995).

Table 4. Effect of protein sources on dry matter intake (DMI), digestibility of dry matter (DMD), organic matter (OMD) and nitrogen (ND) of chickens feed tannin containing or tannin free diet with one of either gelatine, feather meal, fish meal or soya-meat meal included for a 28 days feeding period.

Treatments	DMI (g/day)	DMD (%)	OMD (%)	ND (%)
Gelatine	98.60^{Aa}	65.30^{a}	69.40^{a}	49.00^{a}
Feather meal	102.10^{ab}	64.70^{a}	69.00^{a}	44.30^{A}
Fish meal	107.10^{Bb}	67.30^{a}	72.20^{b}	54.40^{Bb}
Soya-meat	97.80^{Aa}	65.10^{a}	69.50^{ab}	46.00^{A}
SEM	2.30	0.01	0.01	0.02

Values within column followed by different superscript letters are significantly different (capital letters for P<0.01 and small letters for P<0.05).

Table 5. Interaction of protein sources x level of tannins on dry matter intake (DMI), digestibility of dry matter (DMD), organic matter digestibility (OMD) and nitrogen (ND) of chickens fed tannin containing or tannin free diet with one of either gelatine, feather meal, fish meal or soya-meat meal included for a 28 days feedingperiod.

Treatmets	Level of QT (%)	DMI (g/day)	DMD (%)	OMD (%)	ND (%)
Gelatine	0	91.40^{B}	71.40^{B}	75.70^{B}	58.90^{B}
	5	105.70^{A}	59.30^{A}	63.00^{A}	39.00^{A}
Feather meal	0	108.20^{B}	65.50^{a}	69.20^{a}	48.30^{b}
	5	95.90^{A}	64.00^{a}	68.80^{a}	40.30^{a}
Fish meal	0	108.90^{b}	72.40^{B}	77.50^{B}	63.70^{B}
	5	105.30^{a}	62.20^{A}	66.90^{A}	45.10^{A}
Soya-meat meal	0	98.70^{b}	72.70^{B}	76.80^{B}	56.70^{B}
	5	97.00^{a}	57.50^{A}	62.20^{A}	35.40^{A}
SEM		3.30	0.01	0.01	0.02

Values within each protein sources followed by different superscript letters are significantly different (capital letters for P<0.01 and small letters for P<0.05).

The more obvious interaction between tannin and protein sources can be seen in two sources of proteins (gelatine and feather meal) on chicken's performance in general (Table 5). Gelatine significantly elevated dry matter intake on chicken given QT. By contrast, feather meal diets were dramatically reduced the dry matter intake of chickens given QT. The ability of a particular protein to eliminate the effect of tannin in the diet appears to depend on the interaction between the tannin's protein precipitating ability, amino acids profile, protein-tannin ratio, solution pH and ionic strength. The inclusion of gelatine (2.50%) in the diet seems to totally eliminate the adverse effevts of tannin by stimulating the DM intakes. Mehansho *et al*. (1985) reported that inclusion of gelatine in the hamster and rat's diets effectively overcame the growth inhibitation factors of shorgum tannin.

It may be hypothesized that the high proline content of gelatine bound some of the tannins, causing less free tannins that may depress the absorption of nutrients even though there was no improvement in the nitrogen digestibility (Table 5). Gelatine has been reported as a good binder for tannin. From the proline prospective, the feather meal diet had a higher proline content than the gelatine diet (Table 2). However, it might not be possible for proline to be released as a soluble proline enabling binding tannin to the same extent as gelatine does. Alternatively, the existence of proline in gelatinebehaves differently than proline in feather meal for binding tannin. As Mole *at al.* (1990) suggest, tannins may bind selectively to a particular protein in the digestive tract. Tannin preferably binds gelatine even when it comprises only 1% of the protein supplementation. The phenomenon of interaction has also been demonstrated by Mansoori and Acamovic (1998) who found that gelatine reduced the amount offree tannicacid in the digestive tract, resulting in a reduction in the deleterious effects of tannic acid and thus digestible nutrients are freely available for further absorption.

In addition to the proline content, other amino acids such as arginine or histidine are postulated to have a role in overcoming the deleterious effects of tannins. These amino acids are found in relatively high concentrations and therefore intake in the feather meal diet, but again even this high amino acid profile was unable to lift the performance of chickens consuming this diet. This implies that increasing the concentration of these amino acids in the diet is not a useful strategy for overcoming the deleterious effects of tannins in such diets. Other characteristics suchas solubility or digestibility are required. Kawamoto and Nakatsubo (1997) suggested that the ability of a particular protein to precipitate tannin was directly related to itsprotein solubility. Therefore, high profiles of proline, arginine or histidine, along with a high in solubility of proteins is probably the main determinant of the effectiveness of proteins as ameliorants to tannin action.

Conclusions

The present results suggest that 5% of QT in the chickens diet resulted in a depression of the utilization of dietary nutrients by reduing digestibility. Fish meal substantially diminished the adverse effects of QT in digestibilty of nutrients. Relative amelioration effects, in terms of nutrients digestibility, were ranked from the highest to the lowest : fish meal, gelatine, soya-meat meal and feather meal respectively.

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