Utilization of Indigofera (Indigofera zollingeriana) leaf meal in the ration on chemical meat composition, carcass and non-carcass production, and feces-derived methane yield of male growing rabbit

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Livestock and Animal Research
Accredited by Directorate General of Strengthening for Research and Development No. 10/E/KPT/2019

Open Access
Livest. Anim. Res., *July 2022, 20(2): 185-193*
p-ISSN 2721-5326 e-ISSN 2721-7086
https://doi.org/10.20961/lar.v20i2.58270

Original Article

Utilization of Indigofera (*Indigofera zollingeriana*) leaf meal in the ration on chemical meat composition, carcass and non-carcass production, and feces-derived methane yield of male growing rabbit

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Received: January 8th, 2022; Accepted: June 17th, 2022; Published online: July 17th, 2022

Abstrak

Tujuan: Tujuan dari penelitian ini adalah untuk mengevaluasi pengaruh penyertaan tepung daun indigofera (TDI) dalam ransum terhadap produksi karkas non-karkas, kandungan nutrient daging dan produksi methana dari feses kelinci New Zealand White (NZW).
Metode: Penelitian ini menggunakan dua puluh delapan kelinci NZW jantan dengan rerata bobot badan awal 1455.25 ± 142.41 g, dengan umur 65-70 hari. Terdapat empat perlakuan yaitu: pakan basal (T0), penyertaan 4% (T1), 8% (T2) dan 12% TDI pada ransum (T3). Pakan dibuat dalam bentuk pellet, dan iso-protein dengan 16% kandungan protein kasar (PK). Variabel yang diamati meliputi produksi karkas, non-karkas, kandungan nutrien daging dan produksi methan feses kelinci.
Hasil: Hasil penelitian menujukkan bahwa penyertaan TDI pada ransum kelinci sampai dengan taraf 12% tidak berpengaruh nyata (P>0.05) terhadap produksi karkas, non-karkas, kandungan nutrient (PK, lemak kasar (LK), kadar air (KA) dan kadar abu) baik pada otot *Longissimus dorsi* (LD) maupun *Bicep femoris* (BF). Kadar air pada otot LD lebih rendah daripada KA otot BF, sedangkan kadar LK otot LD lebih tinggi daripada kadar LK otot BF. Hasil penelitian juga menunjukkan bahwa penyertaan TDI pada ransum sampai pada taraf 12% tidak berpengaruh nyata (P>0.05) terhadap produksi methan feses kelinci.
Kesimpulan: Perlakuan pada penelitian ini tidak memberikan dampak negatif pada variabel yang diamati, dengan demikian TDI dapat digunakan untuk mensubstitusi bahan pakan ternak kelinci sampai dengan kadar 12%.

Kata Kunci: Daging; Feses; Indigofera; Kelinci; Methan

Abstract

Objective: The aims of this study were to evaluate the inclusion of Indigofera leaf meal (ILM) in the ration on the carcass and non-carcass production, meat chemical composition and feces-derived methane yield of New Zealand White (NZW) rabbits.
Methods: Twenty eights male NZW rabbits with initial body weight of 1455.25 ± 142.41 g, and 65-70 d old were used in this study. There were four treatments namely basal feed (T0); inclusion 4% (T1); 8% (T2), and 12% of ILM (T3) in the ration. The ration was made in form of pellet, and iso-protein

that containing 16% of crude protein (CP). The observed variables were carcass and non-carcass production, meat nutrient content and feces-derived methane production.

Results: The study result showed that inclusion of ILM in the rabbit ration up to 12% gave no significant effect (P>0.05) on the carcass and non-carcass production, nutrient content of the rabbit meat (CP, crude fat (CF), moisture and ash) both in *Longissimus dorsi* (LD) and *Bicep femoris* (BF) muscles. The moisture concentration in LD muscle showed lower than that in BF muscle, while CF content of LD was higher than that in BF muscle. The result also showed that the incorporation of ILM in the ration up to 12% gave no significant effect (P>0.05) on the methane production of rabbit feces.

Conclusions: The inclusion of ILM into diets did not detrimentally affect the observed variables, therefore ILM can be used as a protein source in male growing rabbit ration up to 12%.

Keywords: Feces; Indigofera; Meat; Methane; Rabbit

INTRODUCTION

Feed costs occupy the most expenditure on the livestock industry in developing countries. Martene and Gidenne [1] reported that in rabbit production the feed cost is approximately 70% of the total cost production. This condition is caused by the fact that for some protein source feedstuff in that area must be imported from other countries. The increasing demand of soybean meal (SBM) has increased the price of SBM, this fact has encouraged the researchers to find some alternative feed ingredient to replace SBM [2]. Therefore, utilization of alternative protein source with high nutritional value, environmental sustainability and low production cost are required [3].

Some crops that can grow well in Indonesia have high crude protein (CP) content that potentially be used to replace SBM in animal ration. One of these crops is Indigofera which is classified as a leguminous plant and has capability of producing high quality forage (CP: 27.58%; tannin: 0.08%; saponin: 0.41%; neutral detergent fibre (NDF): 43.56%; acid detergent fibre (ADF): 35.24% [4]. This high nutrient concentration, the fact that Indigofera plants do not compete for human food and can grow well in Indonesia therefore Indigofera is expected to be able to substitute the use of SBM as a protein source in the ration and subsequently can reduce the high animal feed cost.

Rabbit has a big potential as a meat supplier in developing country, since rabbit have low production cost, low investment requirement and low economic risk, as well as it can contribute to improve nutrient family intake, income generation and gender empowerment [5]. Rabbit meat is known as a nutritious and healthy food. Water and protein rabbit meat contents is relatively constant (73.0% and 22%) with high level of essential amino acids. It has low lipid content (1.8%) with mineral content is also constant at around 1.2–1.3%. Moreover, it contains considerably high amount of unsaturated fatty acids (UFA), mainly mono unsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) concentration at around 60.5% [6].

Along with meat production will always be followed by waste production both in the form of solid and liquid waste. With regards to the greenhouse gases (GHGs) emission from the livestock industry, this GHG emissions is originate not only from animals through CO_2 exhalation and CH_4 enteric fermentation but also from manure through the release of CO_2, CH_4 and N_2O [7]. Protocol to calculate methane emissions from animal manure is using organic matter (OM) excreted by the animals, CH_4 conversion factor and ultimate methane yield (B_0) [8]. Therefore, this present study was also aimed to access that information. This is in line with Intergovernmental Panel on Climate Change (IPCC) [9] that recommend expanding the representativeness of the default value data, particularly for livestock in tropical regions that using varying animal diet. Furthermore, a more precise B_0 data will provide substantial information for dimensioning, projecting, and economic budgeting of new biogas plants based on that particular animal manure [8]. This study was aiming to evaluate the effect of partial substitution of SBM with Indigofera leaf meal (ILM) in the ration on chemical meat composition, carcass non-carcass production and feces-derived methane yield of male new zealand white (NZW) growing rabbit.

MATERIALS AND METHODS

Animal and diets

The animal handling and sampling procedures was conducted following the standard procedures of rearing and treating of farm animals stated in law of the Republic of Indonesia number 18, 2009 regarding animal husbandry and health.

Twenty eights NZW buck rabbit at the initial age of about 65-70 d (1455.25 ± 142.41 g) (CV=9.79%) were randomly divided into four groups (each group consisted of 7 replications). There were four treatments namely basal feed (T0); basal feed that partially substituted with ILM at 4% (T1); 8% (T2); and 12% (T3) respectively. The animals were kept in individually housed under similar digestibility cake (wire mesh with bamboo slate: 400 x 600 x 400 mm).

The mature ILM were obtained from local farm in Salatiga. In this recent study, only the leaves part of the plant that use as feed ingredient. The ILM was prepared by sun drying for three days followed by grinding with 0.8 ml screen size. It was kept in sealed plastic and plastic container at room temperature until used for feed ingredient. The animal feed was consisted of concentrate mixture (rice bran, corn, coffee peal, wheat and pollard bran, coconut cake, molasses, mineral, salt, SBM and ILM) and *Synedrella nodiflora.* This plant was used in this study since it has alkaloids, flavonoids, triterpenes, saponins, simple phenolics and polyoses that can act as antidiarrhoea [10]. The concentrate mixture was made in the form of pellets and the *Synedrella nodiflora* was wilted before given to the animals. The ration was made in iso-protein containing about 16% of CP (Table 1). The animals were fed pellets (125 g in total per d) for two times a day at 7.30 am and 4 pm and *Synedrella nodiflora* (10 g) once a day at 4 pm and remained was recorded the next day. Water was accessible at all times using automatic nipple. The chemical composition *Synedrella nodiflora* was dry matter (DM): 36.60%, ash 14.70%, CP: 16.35%, crude fat (CF): 2.38% and crude fibre (CF) 14.40%.

The bucks were adapted for two weeks followed by a week preliminary period and 9 weeks of collection data. The all animals were fed basal ration during adaptation period and it were fed treatment ration during preliminary and collection data period. Feces and urine were collected in the last 10 d of experimental period using collecting tray that fitted to metabolic cage. The collecting tray was made tilted and perforated at the outer end, therefore urine can be funnelled into a bottle containing 10% of sulphuric acid. After weighing, feces were sprayed using 10% sulfuric acid except for the anaerobic digestion (AD) test. The urine and feces were stored frozen until used for chemical composition and AD evaluation test.

Slaughter procedures and sample collection

Following 9 weeks the data collection period, five bucks in each treatment were slaughtered after previously fasted for 12 h with the free access to clean drinking water. Prior to slaughter the rabbits were weighted in which the percentage carcass data were calculated based on this data. Slaughtered rabbits were skinned and then eviscerated. The digestive tract and cecum were separated and weighed. The liver, heart with lungs, legs, and tail were separated. The remaining part was considered as a hot carcass. Classification of rabbit organs in both carcass and non-carcass parts was carried out according to Brahmantyo *et al*. [11]. The hot carcasses were kept at 4 °C for 24 h and referred to as the cold carcass [12]. The *Biceps femoris* (BF) and *Longissimus dorsi* (LD) muscle samples were collected from the cold carcass wrapped using aluminium foil and kept it in sealed plastic at freezer temperature until used for chemical composition analysis. Those two muscles were chosen to represent active and passive muscles respectively.

Feces-derived methane yield

Evaluation to analyse ultimate methane yield (B_0) of rabbit feces was performed by AD batch test using 500 ml infusion bottle [13]. Each reactor contained substrate and inoculum except for control that contained inoculum only. This study using digested slurry of active digester biogas at the Faculty of Animal and Agricultural Sciences, Diponegoro University farm as inoculum. For degassing inoculum, the digested slurry was kept in anaerobic condition at 35 °C for two weeks. The digested slurry was filtered using cloth and only the liquid fraction was subsequently used to inoculate the batch tests. pH, DM/total solid

Table 1. The composition and nutrient content of the ration

Observed variables	Treatments			
	T0	T1	T2	T3
Feed ingredient composition	(%)			
Rice bran	30.00	25.00	25.00	20.00
Corn	20.00	20.00	20.00	17.00
Soy bean meal	20.00	16.00	12.00	8.00
Coffee peal	5.00	5.00	2.00	1.00
Wheat pollard	17.00	15.00	11.00	15.00
Indigofera leaf meal	0.00	4.00	8.00	12.00
Coconut meal	5.00	12.00	19.00	24.00
Molasses	1.00	1.00	1.00	1.00
Salt	1.00	1.00	1.00	1.00
Mineral	1.00	1.00	1.00	1.00
Nutrient content				
Dry matter	92.39	93.91	92.34	93.91
Crude protein	16.18	16.17	16.11	16.10
Crude fat	4.63	4.40	4.89	4.21
Crude fibre	19.11	19.50	21.52	24.47
Gross energy (kkal/kg)	4125.93	4224.20	4251.65	4274.59

(TS) and organic matter (OM)/volatile solid (VS) of filtered inoculum were 7.1, 0.66% and 0.57% respectively. The anaerobic condition inside the digester was obtained by flushing the digester with nitrogen for two minutes. Each bottle was sealed with a rubber stopper and aluminium crimp. The batch digester was kept in the incubator with temperature 35 °C. The gas was measured periodically using the acidified water displacement method [14]. The net methane production from the substrate was calculated as the total gas production from each digester that contained substrate and inoculum, with control subtracted, and it was corrected to STP condition. The AD test was done in four replications and was run for 90 d.

Analytical procedures

The value of pH was measured using a pH meter (Ohaus® ST300 pH meter). Total solid was evaluated by drying samples at 105 °C for 7 h followed by combusting the dried sample at 550 °C for 7 h to measure the ash content. Volatile solid was calculated by subtracting the ash concentration from the DM [15]. CP was analyzed using kjeldhal method while CF was determined using soxhlet extraction method. Energy content of rabbit pellet was determined using bomb calorimeter. Biogas composition was analysed by means of carbon dioxide (CO_2) and methane (CH_4) concentration using Gas Chromatography-Thermal Conductivity Detector (GC C-TCD) Shimadzu 8A [16].

The collected data were analyzed using ANOVA with 95% confidence level manually using Microsoft Excel. Duncan's multiple range tests were used in post ANOVA analysis when significant effect of the treatment was found to be significant on the observed variables [17].

RESULTS

Carcass and non-carcass production

Data about carcass and non-carcass of male NZW rabbit fed inclusion of ILM in the ration with different percentage are given in Table 2 and Table 3. The hot carcass production was in the range of 1292.90-1414.36 g that corresponds to 55.11 to 56.76%. Meanwhile, the cold carcasses were in the range of 1284.38 to 1400.30 g that corresponds to 54.74 to 55.56%. Non-carcass production of growing NZW rabbit fed inclusion of ILM in the ration with different percentage can be seen in Table 3. The non-carcass production weigh was in the range of 43.24% to 44.89%.

Meat chemical composition

The mean muscle nutrient content of BF and LD muscles fed ILM at different levels can be seen in Table 4. The moisture content of BF muscle from all treatments was in the range of

Table 2. Hot and cold carcass weight and its percentage

Observed variables	Treatments			
	T0	T1	T2	T3
Hot carcass (g)	1392.93	1368.54	1414.36	1292.90
Cold carcass (g)	1386.23	1363.58	1400.30	1284.38
Hot carcass percentage (%)	56.38	56.76	56.22	55.11
Cold carcass percentage (%)	56.10	56.56	55.67	54.74

Table 3. Non carcass weight and its percentage

Observed variables	Treatments			
	T0	T1	T2	T3
Non carcass (g)	1081.07	1042.46	1101.64	1047.70
Head (g)	234.08	222.08	236.14	237.55
Tail (g)	13.75	10.53	13.56	13.39
Blood (g)	63.06	69.81	63.23	63.22
Skin (g)	249.19	234.67	259.49	243.09
Leg (g)	62.14	61.87	64.38	66.48
Viscera bruto (g)	459.47	443.51	464.84	422.33
Viscera net (g)	233.31	214.33	235.52	220.50
Percentage				
Non carcass (%)	43.62	43.24	43.78	44.89
Head (%)	21.83	21.33	21.44	22.72
Tail (%)	1.27	1.02	1.23	1.28
Blood (%)	5.72	6.66	5.73	6.07
Skin (%)	23.34	22.45	23.57	23.28
Leg (%)	5.76	5.92	5.85	6.37
Viscera bruto (%)	42.14	42.61	42.18	40.29
Viscera Net (%)	21.48	20.67	21.36	21.06

73.92% to74.47%, CP in the range 21.60% to 21.81%, and CF of BF muscle was in the range of 1.21% to 1.33%. Meanwhile the moisture, CP, and CF content for the LD muscle were in the range of 70.94% to 71.41%, 21.16% to 21.92%, and 2.23% to 2.30% respectively.

Feces-derived methane yield

The methane ultimate yield of rabbit feces from all treatments are presented in Table 5. The mean feces-derived methane yields were 245.59, 250.28, 232.55, and 230.24 ml/kg VS for T0, T1, T2 and T3 respectively, while methane concentrations in the biogas were 64.21, 66.08, 66.89, and 60.16% for T0, T1, T2 and T3 respectively.

DISCUSSION

Carcass production of male NZW rabbit fed inclusion of ILM in the ration (0-12%) in this recent study was in the range 55.11%-56.76% (Table 1). Study from Sutaryo *et al*. [18] showed that carcass production of male NZW rabbit fed inclusion of *Sargassum* sp. seaweed in the ration up to 8% was in the range 55.18% to 56.04%, while study of Wahyono *et al*. [19] found that local rabbit carcass production was 53,04% while carcass production of male NZW was 51.66%. Therefore, the percentage carcass production in this recent study is in accordance with the previous study results.

There was no negative impact (P>0.05) on the inclusion of ILM in the ration up to 12% in the male NZW carcass production. This phenomenon is caused by the fact that there was no effect (P>0.05) of animal slaughter weight among treatment (data not shown). No impact inclusion of ILM in the ration up to 12% on the animal slaughter weight showed that the rabbit has equal capability to utilize nutrient content both in the basal ration and in the treatment ration [18]. de Lima [20] reported that the meat quality is depend on the intrinsic and extrinsic factors. The intrinsic factors are related to animals; therefore, these factors are less variable. The

Table 4. Chemical meat composition

Chemical composition (%)	Treatments			
	T0	T1	T2	T3
Biceps femoris				
Moisture	74.47	74.27	74.21	73.92
Ash	1.17	1.27	1.39	1.26
Crude protein	21.81	21.79	21.79	21.60
Crude fat	1.25	1.21	1.33	1.23
Longissimus dorsi				
Moisture	71.10	70.94	71.41	71.13
Ash	0.96	1.06	1.03	1.08
Crude protein	21.92	21.36	21.16	21.42
Crude fat	2.28	2.23	2.30	2.23

Table 5. Feces quality and feces-derived methane yield

Observed variables	Treatments			
	T0	T1	T2	T3
Crude fat (% DM)	8.14	9.08	9.20	8.64
Crude protein (% DM)	38.47	37.62	36.57	33.48
Methane yield (ml/g VS)	245.59	250.28	232.55	230.24
Methane concentration (%)	64.21	66.08	66.89	60.16

intrinsic factor including breed, sex, age, weight, genes, and type of muscle fiber.

Inclusion of ILM in the ration gave no effect (P>0.05) on the non-carcass production. Non-carcass production in this study was in the range of 43.24% to 44.89%. No inclusion impact of the ILM in the ration on the non-carcass production in this study can be due to the inclusion of ILM in the ration up to 12% gave no effect on feed quality, palatability and digestibility therefore gave no effect of feed treatment on the slaughter weight. Al-Dobaib [21] reported that non-carcass production of four different breed (V-line, Saudi-1, Saudi-2 and, Saudi-3) was in the range 43.2% to 48.4%. Therefore, the result of this study is comparable with the previous study.

Study from Sutaryo *et al.* [18] showed that the LD muscle nutrient content of male growing NZW rabbit fed inclusion *Sargassum* sp. in the ration up to 8% containing 15% CP, was in the range of 20.48%-21.02%, 1,27%-2,97%, 72.46%-73.04%, for CP, CF, and moisture respectively. In addition, study from Paci *et al.*, [22] showed that nutrient content of BF muscle of grey-coloured local (agouti) rabbit that reared at density 2.5/m2 and slaughtered at 103 d old was 22.93%; 1.25%. and 74.59% for CP, CF and moisture respectively. Therefore, the result of this recent study is in comparable with the previous study result.

There was no significant effect (P>0.05) inclusion of ILM in ration up to 12% on the meat chemical composition both of LD and BF muscle of growing NZW rabbits. No significant effect inclusion of ILM on the CP of meat rabbit can be due to the inclusion of ILM in ration in this study resulted no effect on the feed palatability, CP of feed consumption, and CP feed digestion therefore the CP feed absorption was relatively same among the treatment. According to Maharani *et al.* [23] that protein intake has an important role in protein deposition through protein synthesis and degradation. The more protein retained, the better it can contribute to protein deposition, resulting in a higher mass of meat protein. The same phenomenon was also occurring in the CF and ash concentration of rabbit meat. According to Soeparno [24], the CF content of meat can increase if there are additional sources of fat and energy in the animal feed, on the contrary it can decrease if there are additional sources of protein in the animal ration. No significant effect (P>0.05) of ash concentration in the rabbit meat of this recent study can be due to the incorporation of ILM in the rabbit ration did not gave no effect on the ration quality. This fact along with the same feed OM consumption (data was not shown) therefore resulted in no significant effect of the inclusion ILM in the rabbit feed. Brahmantyo *et al*. [25] reported that the ash concentration indicates the mineral content in the feed ingredient, when the feed consumption and feed digestibility are relatively same therefore the ash meat content is also relatively equal between the treatment.

The CF content of meat has a negative relationship with the moisture content of the meat, therefore the higher the CF content of the meat, the lower the moisture content of the meat. Results of this study showed that the CF concentration of the BF muscle was lower than that of LD muscle. This fact was caused by: a) it has a higher moisture content compare to the LD muscle. b) the fact that the two muscles have different functions, BF muscle is an active muscle on other hand LD muscle is a passive muscle which is possible to be used to deposit CF. This fact is in accordance with the results of Ariana and Oka [26] and Dewi *et al*. [27] experiments that CF content in active muscle was lower than that in passive muscle.

Feces-derived methane production of male NZW growing rabbit fed inclusion of ILM in the animal ration was in the range 230.24 to 245.59 ml/kg VS. There was no significant effect (P>0.05) the inclusion of ILM in the animal ration on feces-derived methane production. This fact can be caused by the fact that there was no significant effect the inclusion of ILM on the nutrient (CP and CF) content of animal feces (Table 5). This present study evaluates the effect inclusion of ILM on the methane yield of rabbit feces rather than rabbit manure, since nitrogen in the urine is already hydrolysed to inorganic nitrogen in the animal cage, therefore there will be no methane yield from the animal's urine fraction [8].

Methane production of rabbit feces in this paper was lower than that in the methane production of rabbit feces default for calculation of animal manure methane emissions that is 320 ml/kg VS as reported by IPCC [9]. This phenomenon can be due to the difference of feces nutrient content in those samples since the last data was collected from data greenhouse gas inventory in Italy. In addition, this study was performed in Indonesia, the tropical developing country, therefore this is in appropriate with IPCC [9] recommendation to expand the representativeness of the methane default value, particularly from the tropical regions and when the varying ration is applied.

CONCLUSION

Inclusion of ILM on the growing male NZW rabbit ration up to 12% gave no detrimental impact on the carcass, non-carcass production, meat nutrient content (CP, CF, ash and moisture) of LD and BF muscles. This treatment also gave no impact on methane production of animal feces. Therefore, ILM can be used as a protein source in male growing rabbit up to 12%.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the material discussed in this publication.

ACKNOWLEDGMENTS

The authors thanks to the Diponegoro University for financing this study (Grand Number: 233-100/UN7.6.1/PP/2021).

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