

Increasing dietary crude protein levels attenuates the effects of caecotrope deprivation in growing rabbits

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ABSTRAK

Pengaruh kekurangan caecotrope pada pertumbuhan, metabolisme sekum dan produksi asam lemak volatil (VFA) dipelajari pada kelinci yang diberi berbagai tingkat protein pakan selama percobaan makan 42 hari. Empat puluh delapan kelinci ras campuran (berat awal rata-rata = 875±25 g) dibagi menjadi dua kelompok yaitu; kelompok pemakan caecotrope (CC) dan caecotrope-deprived (CD). Kelinci terbagi dalam 2 kelompok dan masing-masing kelompok diberikan 3 ransum yang mengandung kadar protein kasar (CP) yang berbeda (12, 15 dan 18%) dalam pengaturan faktorial 2 × 3. Hasilnya menunjukkan bahwa peningkatan CP diet meningkatkan ($P<0,05$) caecotrope. Bobot hidup, pertambahan berat badan, rasio konversi pakan, pemanfaatan nitrogen dan produksi VFA pada kelinci CD yang diberi pakan yang mengandung 18% CP sebanding ($P<0,05$) dengan pakan yang diberi CC dengan 15% CP. Pemanfaatan nitrogen meningkat ($P<0,05$) mengikuti peningkatan kadar protein kasar pakan. Proporsi molar asetat, butirrat, dan propionat lebih tinggi ($P<0,05$) pada kelinci CC dibandingkan dengan CD. *Lactobacillus acidophilus* diisolasi dari sekum kelinci CC. Disimpulkan bahwa kekurangan caecotrope memiliki efek merugikan pada pertumbuhan, metabolisme nitrogen, produksi VFA dan bakteri sekum. Efek ini dapat dikurangi dengan meningkatkan tingkat protein pakan pada kelinci yang sedang tumbuh.

Kata kunci: *Caecotrope, Penggunaan protein bakteri sekum, Fermentasi sekum, Asam lemak volatil*

ABSTRACT

The effect of caecotrope deprivation on growth, caecal metabolism and Volatile Fatty acid (VFA) production was studied in rabbits fed varying levels of dietary protein during a 42-days feeding trial. Forty-eight mixed breed rabbits (average initial weight = 875±25 g) were divided into two

groups viz; caecotrope consuming (CC) and caecotrope-deprived (CD) groups. Rabbits in the 2 groups were divided equally and assigned to 3 diets containing different crude protein (CP) levels (12, 15 and 18%) in a 2 × 3 factorial arrangement. The result revealed that increasing dietary CP improved (P<0.05) the proximate composition of the caecotrope. The live weight, weight gain, feed conversion ratio, nitrogen utilization and VFA production in CD rabbits fed diets containing 18% CP were comparable (P<0.05) with those of CC fed diets with 15% CP. Nitrogen utilization increased (P<0.05) following increasing dietary crude protein level. Molar proportions of acetate, butyrate, and propionate were higher (P<0.05) in CC rabbit compared to CD. *Lactobacillus acidophilus* was isolated from the caecum of CC rabbits. It was concluded that caecotrope deprivation has detrimental effect on growth, nitrogen metabolism, VFA production and caecal bacteria and this effect can be attenuated by increasing the dietary protein level in growing rabbits

Keywords: Caecotrope, Caecal bacteria protein utilization, Caecal fermentation, Volatile fatty acid.

INTRODUCTION

The practice of caecotrophy (soft faeces consumption) is one of the most important characteristics of the digestive physiology of the rabbit. It is the strategy the rabbit employs to improve nutrient digestibility and allow maximal utilization and absorption of total ingested nutrients (Tham and Uden, 2013). Caecotropes are packets of partially digested feed, bacteria and bacterial products (including vitamins) eaten directly from the anus (Meredith, 2011). They also contain many microorganisms, which are important for microbial fermentation and the proper functioning of the gut microflora (Kujawinski 2012). Hence, a disorder in the caecotrophy behaviour may have far-reaching consequences on the health and welfare of the rabbit (Wang *et al.*, 2019).

Previous researches (Phiny and Kaensombath, 2006; Wang *et al.*, 2019; Salami *et al.*, 2021) have demonstrated that, caecotrope deprivation leads to malnutrition and reduced growth performance, feed efficiency, lipid synthesis and survivability in the rabbit. It is therefore pertinent that when conditions such as stress, anal injury, sickness and diet imbalance occur, and the rabbit is unable to eat its caecotropes, measures that can cushion the attendant negative consequences must be put in place.

Dietary modification could be a major way of attenuating the negative effects associated with caecotrope deprivation. This is because the rabbit's caecum has the ability to ferment easily available substrate (from any source) for microbial synthesis and protein recycling (Villamide *et al.*, 2010). Suffice to say that an

increase in the levels of available nutrients in the caecum, results in a corresponding increase in the flow of substrates to the fermentative area (de Blas, 2013). Caecotrope deprived rabbits may then be able to make up for lost nutritional benefits by raising the nutrients in diets above recommended levels. However, there exists relatively few known studies on the role of diet in ameliorating the negative effects of caecotrope deprivation in rabbits. Hence, this research aims to determine the effects of increasing dietary protein levels on the growth performance, caecal metabolism and caecal bacteria in caecotrope-deprived rabbits.

MATERIALS AND METHODS

Experimental Location

This study was carried out at the teaching and Research Farm of the Federal University of Agriculture, Abeokuta, Nigeria (7°9'20.56"N, 3°20'42.32"E). This lies in the tropical climate with average annual rainfall of 1100mm and annual mean temperature of 34°C and 80% relative humidity.

Experimental Procedure

A total of forty-eight, 8-week-old mixed breed rabbits with an average initial live weight of 875 ± 25 g were housed individually in well-ventilated stainless steel welded mesh cages (hutches measuring 0.85×0.65×0.50 m in dimension) fitted with feeders and waterers and kept inside well ventilated shed with cemented floor, under controlled light cycle (12 h light/12 h dark). Three isocaloric and iso-fibrous diets with varying crude protein levels (12, 15, and 18%)

were formulated (Table 1) based on the recommendations for minimum and maximum dietary crude protein requirement in grower's mash (de Blas and Mateos., 2010).

After a 7-day acclimatization period, the rabbits were divided into two groups namely caecotrope consuming (CC) and caecotrope-deprived (CD) groups. Rabbits in the CD group were prevented access to their caecotrope with the aid of the plastic neck collar. The flat collar was made out of rigid polypropylene (2mm thick). The mean external diameter averaged 25.0 cm and weighed 50g as described by Gidenne and Lapanouse (2000). The two groups of rabbits were randomly assigned to 3 diets containing different crude protein levels (12, 15 and 18%) in a 2×3 factorial arrangement. The 6 treatment groups consisted of 8 rabbits each, in a completely randomized design. Feed and water were offered *ad libitum* throughout the experimental period. that lasted 42 days.

Sample Collection

During the last 7 days of the trial, 3 rabbits per treatment (18 rabbits in all) were randomly selected from each treatment and confined to

metabolism cages for the total collection of faeces and urine. Rabbits were individually housed in cages that permit the partition of feces and urine. An acclimatization period of 3 days was allowed prior to the total collection of samples, which lasted 4 days. The rabbits were given a known weight of feed throughout the metabolic trial. Hard feces and caecotropes were collected and weighed. Urine was collected individually in clean bottles under 1 M H₂SO₄ (50 ml/L, final pH<3). Urine sample was weighed, diluted (to 1 L), sampled (100 mL) and stored at -20°C till required for analysis. The proximate composition in feeds and feces and nitrogen content of urine was determined according to AOAC (2005).

On day 42 of the experiment, 5 rabbits per treatment (30 rabbits in all) were slaughtered by cervical dislocation, dissected and the caecum excised and weighed. Caecal content pH was measured with a glass electrode pH-meter. Immediately afterwards, two samples of caecal contents were taken (1 g each), acidified with either 0.2 M HCl or 0.5 M H₃PO₄ and both stored at -20°C for ammonia and volatile fatty acid (VFA) determination, respectively. The remaining cae-

Table 1. Ingredients (g/kg) and Chemical Composition (g/kg DM) of the Experimental Diets

	Diet 1	Diet 2	Diet 3
Ingredient (g/kg)			
Maize	280.00	250.00	270.00
Soya bean meal	100.00	170.00	180.00
Fish meal (72% CP)	0.00	0.00	10.00
Wheat offal	300.00	260.00	300.00
Rice husk	150.00	140.00	200.00
Maize offal	130.00	140.00	0.00
Oyster Shell	15.00	15.00	15.00
Bone meal	15.00	15.00	15.00
Vitamin premix	5.00	5.00	5.00
Salt (NaCl)	5.00	5.00	5.00
Total	1000.00	1000.00	1000.00
Determined by Analysis:			
Dry matter (%)	89.90	88.15	90.23
Metabolizable Energy (Kcal/kg)	2500.00	2500.00	2500.00
Crude protein (%)	12.35	14.95	18.88
Crude fibre (%)	7.85	7.31	7.96
Ash (%)	10.04	10.52	10.06
Ether extract (%)	4.45	4.09	4.68
Dry matter (%)	89.90	88.15	90.23

Composition of vitamin-mineral mix per Kg diet: vit A: 400000IU, vit D₃: 800000IU, vit E: 40000mg, vit K: 800mg, vit B₁: 1000mg, vit B₂: 6000mg, vit B₆: 5000mg, vit B₁₂: 25mg, Niacin: 6000mg, Panthothenic acid: 2000mg, Folic acid: 200mg, Biotin: 8mg, Manganese: 300000mg, Iron: 8000mg, Zinc: 20000mg, Cobalt: 80mg, Iodine: 400mg, Selenium: 40mg, Choline: 800000mg, 200 ppm Co (CoSO₄.7 H₂O), 3000mg Cu (CuSO₄.5 H₂O), 20000 mg Fe (FeSO₄.H₂O), 8000 mg Mn (MnO₂), 30000 mg Zn (ZnO), 30 mg Se (Na₂SeO₃), 500 mg I (KI).

cal contents were weighed (20 to 50 g), diluted in a methylcellulose solution (9 g NaCl/L, 1 g methylcellulose/L) and chilled at 4°C for 24 h to dislodge and isolate adherent bacteria as previously described (Belenguer *et al.*, 2005).

Nitrogen (N) was measured by the Kjeldhal method. Total VFA concentrations of caecal content were measured by steam distillation technique according to Eadie *et al.* (1967) while molar percentages of propionic, acetic and butyric acids were determined by using Gas-liquid chromatography (Samuel *et al.*, 1997). Caecal NH₃-N concentrations were measured by spectrophotometry according to Chaney and Marbach (1962).

The bacteria isolated were identified using morphological culture characteristics i.e., colour, consistency, shape, size, elevation, edge, opacity and biochemical test (such as oxalate, citrase, catalase, e.t.c.). Bacteria were identified based on the result obtained from biochemical characterization.

Statistical Analysis

Data were analyzed by ANOVA as a complete randomized design, following a 2 × 3 factorial arrangement, with caecotrope deprivation as factor and dietary crude protein (12%, 15% and 18%) as level. Data were analyzed using the using the General Linear model procedure of the statistical software SAS (2004). The level of statistical significance was set at P<0.05.

Ethics Approval

“All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. In line with the ethics regulations stated in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science (NRC, 2011). The research group ensured all the rats received humane care throughout the period of the study

RESULTS AND DISCUSSION

Effects of dietary protein levels on the proximate composition of soft faeces

Table 2 revealed that the Dry matter (DM), CP, ash and ether extract content of the soft feces increased (P<0.05) following increasing CP level in the diet. This observation is in line with the findings of Rodríguez-Romero *et al.* (2011) who reported that dietary fibre compo-

sition is correlated with the crude protein content of the caecotropes. This finding may be due to the extra nutrients (protein) supply which might have promoted a higher microbial activity and consequently nutrient recycling in soft faeces (García *et al.*, 2002). The rabbit caecum ferments easily available substrate for microbial synthesis and protein recycling (Villamide *et al.*, 2010)

Growth Performance

The trend in result on Table 3 revealed that performance index in CD rabbits fed diets containing 12 and 15% CP were poorer (P<0.05) than those of their CC counterparts. The negative effect of caecotrope deprivation seen in the current study may be due to lesser absorption of vitamins and essential amino acids in microbial cells (Wang *et al.*, 2019). On the other hand, CC rabbits on 15% CP had performance index similar to those of CD rabbits fed diet containing 18% CP. This observation suggests that, feeding crude protein above recommended levels may be beneficial in attenuating the negative effect of caecotrope deprivation on growth and feed utilization. In comparison to rabbits on other dietary treatment, CD rabbits fed diet containing 12% CP had the highest (P<0.05) mortality rate. This suggests that the negative effect of caecotrope deprivation is more pronounced under poor dietary conditions. Higher mortality resulting from poor diet could be explained by the fact that caecotrope deprivation reduces the population of intestinal bacteria. This has negative consequences on metabolism, immune system development, disease resistance and other physiological functions necessary for the health and survivability of the rabbit (Heinken *et al.*, 2013; Goto *et al.*, 2015). Reduced mortality rate seen in CD rabbits fed diets containing 18% CP suggests that an extra supply of dietary protein might be necessary for attenuating the negative consequences of caecotrope deprivation on intestinal flora.

Nitrogen Utilisation

Results on Table 3 revealed that caecotrope consumption and increasing dietary crude protein level significantly (P<0.05) affected the nitrogen intake, nitrogen absorption and nitrogen retention. The nitrogen intake, percentage nitrogen absorbed and retained increased (P<0.05) following increasing levels of dietary CP in CD rabbits. This observation agrees with the findings of Jegsai *et al.* (1985) who reported that the effect of caecotrophy on protein and amino acid

Table 2. Effects of Dietary Crude Protein Level on the Proximate Composition of Caecotropes

Proximate composition	Dietary crude protein levels			SEM
	12%	15%	18%	
Dry matter (%)	30.58 ^b	30.86 ^b	32.86 ^a	0.19
Crude Protein (%)	31.45 ^b	31.57 ^b	33.03 ^a	0.12
Crude Fibre (%)	15.31	15.54	15.80	0.52
Ash (%)	9.12 ^b	9.26 ^b	10.60 ^a	0.02
Ether extract (%)	2.93 ^b	3.00 ^b	4.20 ^a	0.03

^{ab} Means along the same row with different superscripts are significantly ($P < 0.05$) different
SEM: Standard error of mean

metabolism is greater in rabbits on a poor diet that is deficient in amino acid than with higher protein content. Takahashi and Sakaguchi (2002), also found that the prevention of coprophagy in guinea pig, lowered nitrogen retention and faecal concentration. Caecotrope-deprived rabbits fed diets containing 18% CP and CC rabbits fed diets containing 15% CP recorded similar values for percentage nitrogen absorbed and retained. This observation suggests a cushioning of the effect caecotrope deprivation following increasing dietary crude protein levels.

Characterization of Caecal Fermentation

The practice of caecotrophy and dietary crude protein level (Table 4) showed significant ($P < 0.05$) effects on total volatile fatty acid, propionate, ammonia nitrogen production, caecal pH, acetate and butyrate production and propionate-butyrate ratio. An increase in VFA production and molar proportion was recorded following increasing levels of dietary crude protein and practice of caecotrophy. This may be due to the fact that caecotropes contains bacteria (hydrolytic species) which are involved in the breaking down of complex polymers to smaller compounds (monosaccharides, amino acids, e.t.c.) which are then converted to energy sources (VFA). Increasing dietary protein levels also increased ($P < 0.05$) the amount of substrate used by caecal bacteria for fermentative activities. The highest molar proportion of VFA seen in CC rabbits fed diet containing 18% CP may also be linked to increase levels of available substrates (dietary protein) for fermentation (de Blas, 2013). The caecal pH and ammonia concentration, were similar to those previously reported by Kimsé *et al.* (2012). Compared to rabbits on other treatments, CC rabbits on 18% CP

had the highest ($P < 0.05$) values for pH and ammonia Nitrogen while those in the same group fed diets containing 15% CP had the lowest ($P < 0.05$) values. This observation may be due to the influence of caecotrophy and dietary protein levels on the rabbit caecal ecosystem (Kimsé *et al.* 2012).

Caecal Bacteria

Table 5 shows the different types of bacteria isolated from the caecum of the rabbits fed experimental diets and population counts. The result showed that total viable bacteria count significantly ($P < 0.05$) increased following an increase in dietary CP level and caecotrope consumption. Highest population count observed caecotrope-deprived rabbits fed diet containing 12% CP may be due to proliferation of pathogenic organisms such as *Clostridium perfringens* in the caecum. This may also explain the higher mortality rate seen in this group of rabbits.

Predominant bacterial community seen across treatment groups were firmicutes (*Ruminococcus*, *Clostridium*, *Streptococcus*) and *Bacteroidetes* (Bacteroides). This is consistent with the findings of Combes *et al.* (2011) and Zhu *et al.* (2015). Firmicutes have their importance as a commensal in gastrointestinal health and have been described as the most abundant phylum in the gastrointestinal flora of most healthy mammal. The caecal bacteria of the rabbit change and adapt rapidly to reach a new equilibrium in response to nutrition (Michelland *et al.*, 2011; Vantus *et al.*, 2014). Since caecotropes are a source of nutrients, the variety of bacterial organisms isolated from the caecum of caecotrope consuming and caecotrope deprived rabbits on diets containing varying levels of crude protein may imply that, caecotrophic behaviours

Table 3. Effect of Caecotrophy and Dietary Crude Protein Level on Growth Performance of Rabbits

Caecotrophy	Caecotrope-deprived			Caecotrope-consuming			Significance			
	Dietary CP level	12%	15%	18%	12%	15%	18%	Dietary protein	Caecotrophy	Dietary protein × caecotrophy
Initial weight (g)	12%	900.00	850.00	866.67	833.33	833.33	750	57.74	NS	NS
Final weight (g)	12%	1326.67 ^d	1490.00 ^e	1760.00 ^a	1686.67 ^b	1766.67 ^a	1816.67 ^a	66.05	**	*
Daily weight gain (g)	12%	7.63 ^e	11.60 ^d	15.95 ^{bc}	15.24 ^c	16.67 ^b	19.05 ^a	1.12	**	**
Feed intake (g/d)	12%	36.67 ^d	41.67 ^c	46.67 ^b	41.67 ^c	53.33 ^a	56.67 ^a	2.64	**	**
Feed conversion ratio	12%	4.82 ^d	3.75 ^e	3.01 ^{ab}	2.74 ^a	3.22 ^b	2.97 ^a	0.32	*	*
Protein efficiency ratio	12%	0.52 ^{ab}	0.58 ^a	0.47 ^c	0.50 ^b	0.34 ^d	0.53 ^a	0.04	*	*
Mortality (%)	12%	5.00 ^e	2.17 ^d	0.00 ^a	2.50 ^c	0.00 ^a	1.13 ^b	0.15	*	*

^{abcde} Means along the same row with different superscripts are significantly (P<0.05) different
SEM: standard error of means; NS: (not significant) refers to P>0.05; *P<0.05 (significant at 5%); **P<0.01 (significant at 1%)

Table 4. Effects of Dietary Crude Protein Level and Caecotrophy on Nitrogen Utilization

Caecotrophy	Caecotrope-deprived			Caecotrope-consuming			Significance			
	Dietary CP level	12%	15%	18%	12%	15%	18%	Dietary protein	Caecotrophy	Dietary protein × caecotrophy
Diet CP (%)	12%	12.35	14.95	18.88	12.35	14.95	18.88	0.11	NS	NS
Nitrogen intake	12%	0.72 ^d	1.03 ^c	1.33 ^b	0.82 ^d	1.32 ^b	1.62 ^a	0.07	**	**
Faecal Nitrogen	12%	0.18	0.23	0.24	0.16	0.21	0.19	0.02	*	*
Urine Nitrogen	12%	0.02	0.03	0.02	0.03	0.03	0.04	0.004	*	NS
Nitrogen absorbed	12%	0.54 ^d	0.78 ^c	1.08 ^b	0.64 ^{cd}	1.11 ^b	1.42 ^a	0.07	*	**
% Nitrogen absorbed	12%	74.29 ^c	75.50 ^c	80.28 ^b	76.75 ^{bc}	81.49 ^b	88.01 ^a	2.54	*	*
Nitrogen retained	12%	0.52 ^c	0.75 ^c	1.06 ^b	0.61 ^d	1.08 ^b	1.38 ^a	0.07	*	*
% Nitrogen retained	12%	71.51 ^c	72.89 ^a	80.22 ^b	73.43 ^c	81.52 ^{ab}	85.56 ^a	2.63	*	*

^{abc} Means along the same row with different superscripts are significantly (P<0.05) different
SEM: standard error of means; NS: (not significant) refers to P>0.05; *P<0.05 (significant at 5%); **P<0.01 (significant at 1%)

Table 5. Effect of Caecotrophy and Dietary Crude Protein Levels on VFA Production

Caecotrophy	Caecotrope-deprived						Caecotrope-consuming			SEM	Dietary protein	Significance	
	12%	15%	18%	12%	15%	18%	Caecotrophy	Dietary protein × caecotrophy					
Total VFA (Mm/Litre)	69.77 ^e	67.17 ^e	81.93 ^b	67.27 ^e	81.63 ^b	94.27 ^a	2.15	*	**	**			
Acetate (C ₂) (mol/100mol)	43.93 ^b	42.87 ^b	52.40 ^a	44.23 ^b	49.73 ^a	52.47 ^a	1.53	*	*	*			
Propionate (C ₃) (mol/100mol)	12.57 ^b	12.67 ^b	12.60 ^b	9.10 ^c	13.23 ^b	23.40 ^a	0.64	*	*	*			
Butyrate (C ₄) (mol/100mol)	9.27 ^b	9.23 ^b	9.07 ^b	9.80 ^b	10.63 ^b	15.43 ^a	1.39	*	*	**			
Propionate: Butyrate	0.74 ^b	0.72 ^b	0.72 ^b	1.03 ^a	0.80 ^b	0.66 ^c	0.09	*	*	*			
pH	5.48 ^{bc}	5.69 ^b	5.47 ^{bc}	5.24 ^c	4.78 ^d	5.94 ^a	0.16	*	*	*			
NH ₃ -N	5.36 ^d	5.74 ^c	6.43 ^b	5.95 ^c	5.15 ^d	7.00 ^a	0.11	*	*	*			

abcd Means along the same row with different superscripts are significantly (P<0.05) different
SEM: standard error of means; NS: (not significant) refers to P>0.05; *P<0.05 (significant at 5%); **P<0.01 (significant at 1%)

and dietary protein results in the proliferation of different types of bacteria in the gut. The activity of diverse bacteria present in caecum and soft faeces of rabbits has a great impact on the overall health status and growth performance of the rabbits as well as imparts on the digestion process in the animals (Combes *et al.* 2013; Kylie 2018). Ureolytic bacteria (*Ruminococcus albus*, *Bacteroides ruminicola*) are responsible for hydrolysing urea in the caecum and play important roles in the nitrogen metabolism of the host (Cheng and Cord-Ruwisch, 2013). *Lactobacillus acidophilus* isolated from the caecum of caecotrope consuming rabbits fed diets containing 12% CP is rarely isolated from the caecum of rabbits. It helps to stimulate immune function by aiding gut microflora in via the production of lactic acid which aids normal bacteria and kills pathogenic bacteria (e.g. *E. coli*) (Fijan, 2014). This observation may suggest that caecotrophy is a form of adaptation, during times when diets are low in crude protein.

CONCLUSION

The observation of the current study suggest that it is unnecessary to feed caecotrope consuming rabbit in excess of their required CP levels (15%) as this level is considered optimum for the rabbit nutrition. However, increasing dietary CP level attenuated the detrimental effects of caecotrope deprivation on the growth performance, caecal fermentation and gut bacteria.. Therefore, caecotrope consumption is a form of adaptation particularly in times of nutritional adversities such as when diet is low in CP.

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