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### Effect of Adding Sodium Nitrate without or with some Feed Additives in Growing Rabbit Diets on: 2. Nutrients Digestibility, some Caecum Fermentation and some Blood Constituents

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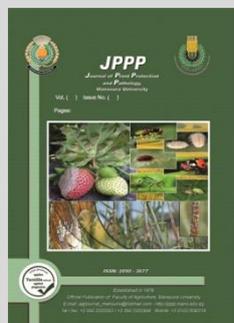
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#### ABSTRACT

Thirty, 14 weeks of age New Zealand White (NZW) rabbits with an average live body weight (LBW) of 2.0 Kg were divided into 10 similar groups (3 in each). The rabbits were housed in a separate cage (3 rabbits in each). Ten pelleted experimental diets were contained two levels of sodium nitrate (0 and 2%). The other four tested feed additives were sodium sulfate, clay, yeast and prebiotic. Diets refers to as R2, R3, R4 and R5 were without sodium nitrate, but with feed additives, sodium sulphate, clay, yeast and prebiotic, respectively. While, diets R6, R7, R8, R9 and R10 were with sodium nitrate and R6 without feed additives but R7, R8, R9 and R10 were with the same feed additives, sodium sulphate, clay, yeast and prebiotic, respectively. The DCP % was higher with feeding on R1, R2 and R7, TDN % was higher with feeding with R1, DCP intake g/d with R1, R5 and R7, TDN intake g/d with R1, DE Kcal/Kg was higher with feeding on R4 and R9, DE intake Kcal/d with R1 and R4 while DEI/DCPI was increased with feeding on R4, R6 and R9. The effect of the feed additives, also showed that the total VFA increased ( $p < 0.05$ ) without feed additives or with added  $\text{Na}_2\text{SO}_4$  or clay than added prebiotic. The highest values were observed with feeding on R1, R2 and R7. The highest values of  $\text{NH}_3$  concentrations were observed with feeding on R3 and R8 diets. Protein concentration was higher with feeding on R4 or R10 than the others. Urea - N concentration was the highest with feeding on R7, while the creatinine concentration was increased with feeding on R9 compared with the other diets. When using the feed additives e.g. prebiotic without sodium nitrate (R4) or with sodium nitrate (R9) caused improving effect on the feeding values of tested diets. However, diets R1 and R6 without or with sodium nitrate respectively showed the best results in case of nutrient digestibility and feed values as well as caecum fermentation and some blood parameters.

**Keywords:** digestibility, rabbits, caecum fermentation.



#### INTRODUCTION

Tadele and Amha (2015) showed that the slow release of nitrogen from the biuret is better proportional to the energy in the diet of livestock consuming low-grade fodder, thus improving feed use and lowering the metabolic cost of eliminating excess N in urea - existing diet.

Nitrates taken up as of the soil via plant roots are usually integrated into plant tissues such as amino acids, proteins, and additional nitrogenous compounds. The principal site for exchanging nitrates into vegetable foodstuffs is the green leaf cultivation activity. Nitrates build up in the stem or stem of plants what time factors impede with natural plant developments.

Necessities for fermentable N for a hypothetically 100 % resourceful rumen fermentation (microbial intensification is most advantageous) is roughly 30 g N/kg organic material digested or 15 g N/kg dry material ingestion at 50 % digestibility. Commencing these calculations, it's come into view that nitrate could reinstate all the urea in a diet wherever animals are become accustomed to the nitrate lacking creating nitrite toxicity.

Nitrate itself is not predominantly poisonous to animals. Nitrates inspired by ruminants are on the whole

condensed to  $\text{NH}_3$  and then engrossed and excreted as urea in the urine or transformed by bacterial into bacteria protein. Nitrite, individual of the transitional products, is the foundation of "nitrate poisoning". A number of the nitrate is engrossed into the blood, wherever it modifies the red decorated hemoglobin to methemoglobin. Hemoglobin brings oxygen as of the lungs to additional tissues, other than methemoglobin cannot bear oxygen. Nitrate turn out to be toxic once methemoglobin creation is high adequate. That the oxygen transportation capacity of the blood is condensed to a serious level. If sufficient methemoglobin is formed, that animal resolve die. The toxic rank depends mutually upon how a large amount and how speedy nitrate was inspired. Eating small amounts of high nitrate feed increases the total amount of nitrate that can be consumed daily by livestock without adverse effects and helps livestock to adapt to high nitrate feed (Rasby *et al.*, 2014).

There is research on anaerobic systems other than rumen indicating that the accumulation of nitrates is powerfully influenced through the population density of specialized microbes with the aim of reduce nitrates to nitrites and oxidize sulphide to sulphate as they increase nitrites to ammonia. Such a rumen reaction from animals on an elevated

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nitrate / protein diet might explain nitrate toxicity pattern in ruminants.

Krasicka *et al.* (1999) demonstrated that microbial protein production in lambs feed meals holding low ranks of cellulose enlarged when sulfur level was increased from 0.2% to 0.8% in the dry matter of the diets. For that reason, complementary of organic or inorganic S complexes in diets holding high ranks of NPN is of grand magnitude in ruminant diet.

Ayyat *et al.* (2000) found that the supplementing with natural clay (bentonite) in rabbit diets contaminated with the pesticide decreased the mortality rate (3.3 % Vs. 16.7 %). Separately from the mycotoxin compulsory capacity, clay raw materials show additional activity which could completely affect animal wellbeing and productivity (Nadziakiewicz *et al.*, 2019).

Probiotics are nutritional supplements for live microbes that constructively affect the crowd by humanizing the balance of intestinal microflora (Girard *et al.*, 1993). A number of benefits have been reported to swallow probiotics. The use of prebiotics in association with useful probiotics may be a worthwhile approach, as the prebiotics preferentially stimulate some probiotic strains (Gibson *et al.*, 2004). Combination of probiotic and prebiotics of symbiotic also enhance probiotic effectiveness.

The current study was conducted to investigate the effect of feeding diets with or without sodium nitrate by adding sodium sulfate, clay, yeast and prebiotics on nutrients digestibility, caecum fermentation and some blood parameters of growing rabbits.

## MATERIALS AND METHODS

The experimental field of this study was conducted at the Experimental Station of the Poultry Production Department, Faculty of Agriculture, Mansoura University. The chemical analysis for experimental diets, faces and blood constituent's measurements were running at the Laboratory of Regional Center for Food and Feed, Agriculture Research Center, Cairo, Egypt.

### Experimental animals and management:

Thirty, 14 weeks of age New Zealand White (NZW) rabbits with an average live body weight (LBW) of 2.0 Kg were divided into 10 similar groups (3 in each). The rabbits were housed in a separate cage (3 rabbits in each). All groups had approximately equal means of live body weight. The dimensions of each cage were 50, 50, 45 cm for length, width and height, respectively. The cages were supplied by a feeder and a stainless steel nipple for drinking.

### Feed additives:

The current research was conducted to assess the effect of inclusion the basal diet with / without sodium nitrate. The experimental basal diets were added with sodium sulphate, clay, yeast culture and prebiotic. Sodium sulphate was obtained from El-Gamhoria company "Mansoura chemical branch" at the Chest Hospital in Mansoura. The clay or "bentonite" was obtained from Sinai Manganese Company, Cairo Egypt. Bentonite contained the following oxides, SiO<sub>2</sub> 49-55 %; Al<sub>2</sub>O<sub>3</sub> 20-24 %; Fe<sub>2</sub>O<sub>3</sub> 2.6-6 %; CaO 0.2-6 %; Na<sub>2</sub>O 1.1-24 %; Mg 0.5-2 % and K<sub>2</sub>O 1.2-1.4 %. Yeast culture is "Progut – a new generation" yeast product. In trials, the application of progut in poultry feeds has led to improved vitality, feed utilization, better productivity and growth. The

perfect (prebiotic) is a buffered blend of specific acids which were with fructo – oligosaccharide (FOS) to promote a healthy gut microflora, which 2 Kg were added to ton feed of the basal diet at feeding time.

### Experimental diets and design:

The Experimental diets were formulated to provide adequate energy and protein for growing rabbits. Ten experimental diets were formulated to be more than 16 % protein according to the (NRC, 1977) recommendations. The constituents of the experimental basal diet were as shown in Table 1. All diets were in pelleted form.

Ten pelleted experimental diets were contained two levels of sodium nitrate (0 and 2%) (Rasby *et al.*, 2014). However, the four feed additives were sodium sulfate (0.2%), clay (2%), yeast (0.25 %) and prebiotic (0.2%).

**Table 1. Ingredients of the experimental basal diets.**

Feed ingredients	Basal diet
Alfalfa hay	32.00
Yellow corn	10.00
Barley	13.00
Wheat bran	20.00
Soybean meal	13.00
Mint	6.15
Aniseed	1.00
Molasses	2.00
Limestone	1.00
Dicalcium phosphate	1.00
Sodium chloride	0.40
Vit. Min. premix*	0.30
Coccdan	0.05
Methionine	0.10

\* Each 2 Kg of premix contains: A: 10.000000 IU; D<sub>3</sub>: 2000000 IU, E: 10000 mg; Zn: 3000 mg, Mn: 2000 mg; Fe: 4000 mg; Cu: 1000 mg; I: 100 mg; Se: 10 mg; Co: 10 mg; Na: 23000 mg; and Mg: 2000 mg; CaCo<sub>3</sub>: added to 2.0 kg.

### The experimental diets were as follow:

R1 = the experimental basal diet (without NaNO<sub>3</sub> or feed additives)

R2 = R1 + 0 NaNO<sub>3</sub>+ 0.2% Na<sub>2</sub>SO<sub>4</sub>

R3 = R1 + 0 NaNO<sub>3</sub>+ 2% Clay

R4 = R1 + 0 NaNO<sub>3</sub>+ 0.25% Yeast

R5 = R1 + 0 NaNO<sub>3</sub>+ 0.2 Prebiotic

R6 = R1 + 2% NaNO<sub>3</sub>

R7 = R1 + 2% NaNO<sub>3</sub>+ 0.2% Na<sub>2</sub>SO<sub>4</sub>

R8 = R1 + 2% NaNO<sub>3</sub>+ 2% Clay

R9 = R1 + 2% NaNO<sub>3</sub> 0.25% Yeast

R10 = R1 + 2% NaNO<sub>3</sub>+ 0.2 Prebiotic

### Digestibility trials:

Rabbits were housed individually in metabolic cages. All rabbits were given their daily feed allowances at 10 am. Drinking water was available at all time. Quantitative collection of feces started 24 hrs. after feeding. The feces of each rabbit were collected every day in the morning. Feces through 1 mm screen, then complete drying was undertaken at 105 °C for 3 hrs. and weighted and stored in tight bottles for chemical analysis.

### Chemical analysis and procedures:

Diets and feces were analyzed for dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), ash, fiber fractions (NDF, ADF ADL, hemi. and cell.) according to A.O.A.C. (1990) and conversion factors for TDN were using McDonald *et al.* (1973), while DE (kcal / kg DM) by : [DE (kcal/kg DM) = (5.28 × DCP) + (9.51 × DEE) + (4.20 × DCF) + (4.20 × DNFE)], (Schiemann *et al.*, 1972).

Non fiberous carbohydrates (NFC) % (DM basi) = OM% - (CP % + NDF % + EE %), (Calsamiglia *et al.*, 1995).

**Parameters related to fermentation in the caecum:**

Caecum fluid samples were collected from tree rabbits chosen randomly from each experimental trials. The samples filtered through two layers of surgical gauze and were used for determining.

**pH:** The pH value was read immediately using battery operated pH meter.

**Total VFA:** After acidification of samples of caecum liquor, the total VFA were steam-distilled from a known volume of sample using the micro-kjeldahl apparatus. The concentration of VFA was calculated by the method of (Abou-Akkada and El-Shazly, 1964).

**Ammonia – N:** The caecum ammonia-N was determined according to the method of Conway and O'Malley (1942).

**Chemical composition of the experimental diets is presented in Table (2):**

**Table 2. Chemical composition of the experimental diets**

Items	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
NaNO <sub>3</sub>	0.0			2%						
Additives	Non	Na <sub>2</sub> SO <sub>4</sub>	Clay	Yeast	Prebiotic	Non	Na <sub>2</sub> SO <sub>4</sub>	Clay	Yeast	Prebiotic
DM	88.9	89.86	89.85	90.00	90.06	90.53	91.10	89.11	91.12	91.06
Composition of DM%:										
OM	90.10	89.01	88.11	88.80	90.06	88.10	84.79	88.56	88.99	87.88
CP	19.46	19.69	19.80	19.23	19.58	18.38	19.87	18.98	18.34	18.90
CF	12.77	18.42	15.10	15.93	18.38	16.11	16.78	18.02	17.01	18.05
EE	4.83	1.24	1.76	1.77	2.33	2.15	1.82	2.52	2.12	2.68
NFE	53.05	49.67	51.45	51.87	49.78	51.46	46.31	49.04	51.53	48.24
Ash	9.90	10.99	11.89	11.20	9.94	11.90	15.21	11.44	11.01	12.12
Fiber fractions %:										
NDF	28.41	32.74	29.43	31.36	30.60	31.95	29.74	30.96	31.63	30.89
ADF	17.60	20.15	18.58	19.12	18.91	19.22	18.09	19.21	20.03	19.27
Hemicellulose	10.81	12.59	10.85	12.23	11.69	12.73	11.65	11.75	10.60	11.62
ADL	4.84	5.46	5.34	5.19	5.19	5.45	7.49	5.85	5.71	5.71
Cellulose	12.77	14.69	13.23	13.93	13.72	13.77	10.60	13.37	14.32	13.56
NFC*	37.40	35.34	37.13	36.44	37.55	35.62	33.36	36.10	36.91	35.41
NFC/NDF	1.31	1.07	1.26	1.16	1.22	1.11	1.12	1.16	1.16	1.14

\*Non fiberous carbohydrates% = OM% - (CP%+NDF%+EE%), (Calsamiglia *et al.*, 1995).

**Blood samples:**

Blood samples were taken at 14 weeks of age from 3 rabbits which were randomly selected from each treatment. 2mls of blood was collected without anticoagulant into sterile test tube for determination serum biochemical indices. The tube containing blood was placed in slanting position at room temperature for clotting. Blood samples were centrifuged at 3000 rpm for 10 minutes and thereafter stored at - 20°C to determine the concentration of serum constituents. Serum protein was measured according to (Dumas *et al.*, 1981); albumin was measured by Hill and Well (1983); globulin, (calculated by difference between the total proteins and albumin concentration); Serum aspartate aminotransferase (AST) and alaninaminotransferase (ALT) were determined according to (Reitman and Frankel, 1957); urea-N, (Freidman *et al.*, 1980), while glucose determination in whole blood by Gluco-tek (Skylar *et al.*, 1981). Alkaline phosphates (ALP), Schumann *et al.* (2011). Creatinine, (Fabiny and Ertinshausen, 1971). Glutamyl transferase (GGT), Li, *et al.* (2011) and malondialdehyde (MDA), Zeb and Ullah, (2016).

**Statistical analysis:**

Data were statistically analyzed by SAS (2000), while the differences among means were tested using Duncan's Multiple Range Test (Duncan, 1955).

**RESULTS AND DISCUSSION**

The constituents of the experimental basal diet are shown in Table (1). The determined values of the chemical composition (% DM) are presented in Table (2). The diets from R<sub>1</sub> to R<sub>5</sub> were without NaNO<sub>3</sub> and without any additives to (R<sub>1</sub>), but R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>5</sub> were given Na<sub>2</sub>SO<sub>4</sub>, clay, yeast and prebiotic, respectively. The diets from R<sub>6</sub> up to R<sub>10</sub> were fed with NaNO<sub>3</sub> and R<sub>6</sub> was without any supplements, but from

R<sub>7</sub> to R<sub>10</sub> diets were fed with the same supplements like diets from R<sub>2</sub> to R<sub>5</sub>.

Hillyer *et al.* (1997) showed that the rabbits diet is pelleted, it is made up of various ingredients specially formulated to provide the nutrients required by rabbits. The typical ingredients of basal diets include alfalfa hay, grain and grain by-products, protein supplements, vitamins and minerals. Table (2) shows the determined analysis of the experimental diets. The results ranged for: CP (18.34 to 19.87 %), CF (12.77 to 18.42 %), EE (1.24 to 4.83 %), NFE (46.31 to 53.05 %), NDF (28.41 to 32.74 %), ADF (17.60 to 20.03 %), hemicellulose (10.60 to 12.73 %), cellulose (10.60 to 14.69 %) and NFC/NDF ratio (1.07 to 1.31). These chemical nutrient were suitable for formulation of growing rabbit's diets as recommended by Gidenne, (1992) and De Blas *et al.*, (1995).

Tables (3 and 4) showed the dry matter intake, nutrient digestibility, digested nutrients and feeding values of the experimental diets. The effect of feeding tested diets without or with added NaNO<sub>3</sub> showed that, the DMI (g/h/d), nutrient digestibility (%) of OM, CP, hemicellulose and cellulose were higher (p < 0.05) when feeding on diet without NaNO<sub>3</sub> than with it, while the digestibility of CF and NFE were higher (p<0.05) with feeding with NaNO<sub>3</sub>. The effect of feed additives showed that the DMI g/d was higher (p < 0.05) with added clay than the others and the lower (p < 0.05) value was recorded with Na<sub>2</sub>SO<sub>4</sub>. The digestibility of DM, OM and CP were higher (p < 0.05) when feeding diet without feed additives or with added Na<sub>2</sub>SO<sub>4</sub> but without significant effect with added yeast on DM and OM digestibility. The digestibility of EE was higher with feeding (p < 0.05) on diet without additives or with add prebiotic than the other additives as shown in Table (3).

The interaction among feeding the experimental diets without or with NaNO<sub>3</sub> and without or with feed additives showed that there were no significant effects on nutrients digestibility, digested nutrients and feeding values as shown in Table (4).

**Table 3. Effect of feeding experimental diets without or with NaNO<sub>3</sub> and without or with feed additives on nutrient digestibility and feeding values**

Items	NaNO <sub>3</sub>		± SEM	Additives					± SEM
	0 %	0.2 %		Non	Na <sub>2</sub> SO <sub>4</sub>	Clay	Yeast	Prebiotic	
DM intake (g/h/d)	144.88 <sup>a</sup>	143.52 <sup>b</sup>		144.92 <sup>b</sup>	143.69 <sup>c</sup>	145.29 <sup>a</sup>	143.56 <sup>d</sup>	143.56 <sup>d</sup>	
Nutrient digestibility (%):									
DM	70.29	69.67	0.345	71.32 <sup>a</sup>	71.18 <sup>a</sup>	68.71 <sup>bc</sup>	70.19 <sup>ab</sup>	68.50 <sup>c</sup>	0.545
OM	72.28 <sup>a</sup>	71.15 <sup>b</sup>	0.319	73.03 <sup>a</sup>	72.36 <sup>a</sup>	70.68 <sup>b</sup>	72.02 <sup>ab</sup>	70.48 <sup>b</sup>	0.504
CP	79.30 <sup>a</sup>	77.99 <sup>b</sup>	0.282	80.24 <sup>a</sup>	79.79 <sup>a</sup>	77.55 <sup>b</sup>	78.12 <sup>b</sup>	77.52 <sup>b</sup>	0.446
CF	38.08 <sup>b</sup>	40.43 <sup>a</sup>	0.682	35.02 <sup>c</sup>	44.31 <sup>a</sup>	34.45 <sup>c</sup>	40.80 <sup>b</sup>	41.71 <sup>ab</sup>	1.079
EE	70.79	75.84	1.708	83.28 <sup>a</sup>	69.28 <sup>b</sup>	64.64 <sup>b</sup>	69.26 <sup>b</sup>	80.11 <sup>a</sup>	2.701
NFE	80.82	79.87	0.395	80.74 <sup>ab</sup>	80.49 <sup>ab</sup>	80.98 <sup>a</sup>	80.66 <sup>ab</sup>	78.84 <sup>b</sup>	0.625
NDF	42.11	40.49	1.586	44.75	42.44	38.35	43.68	37.27	2.508
ADF	33.95	31.55	2.416	37.05	33.17	28.92	35.16	29.45	3.821
Hemicellulose	60.27 <sup>a</sup>	51.24 <sup>b</sup>	1.828	60.14	57.08	54.19	57.34	50.01	2.89
Cellulose	37.48 <sup>a</sup>	33.10 <sup>b</sup>	0.745	34.87 <sup>b</sup>	32.43 <sup>b</sup>	35.17 <sup>b</sup>	40.05 <sup>a</sup>	33.93 <sup>b</sup>	1.178
NFC*	93.25 <sup>b</sup>	95.18 <sup>a</sup>	0.552	94.22	95.27	94.05	93.37	94.16	0.873
Digested nutrients and feeding value as DM (%):									
DCP	15.50 <sup>a</sup>	14.80 <sup>b</sup>	0.074	15.35 <sup>b</sup>	15.78 <sup>a</sup>	15.03 <sup>ab</sup>	14.68 <sup>c</sup>	14.92 <sup>c</sup>	0.117
DCF	6.28 <sup>b</sup>	6.93 <sup>a</sup>	0.114	5.11 <sup>d</sup>	7.81 <sup>a</sup>	5.76 <sup>c</sup>	6.72 <sup>b</sup>	7.60 <sup>a</sup>	0.181
DEE	4.11 <sup>a</sup>	3.86 <sup>b</sup>	0.08	6.73 <sup>a</sup>	2.46 <sup>d</sup>	3.20 <sup>c</sup>	3.01 <sup>c</sup>	4.52 <sup>b</sup>	0.126
DNFE	41.35 <sup>a</sup>	39.39 <sup>b</sup>	0.193	42.19 <sup>a</sup>	38.62 <sup>c</sup>	40.71 <sup>b</sup>	41.70 <sup>a</sup>	38.64 <sup>c</sup>	0.305
TDN	67.24 <sup>a</sup>	64.98 <sup>b</sup>	0.281	69.37 <sup>a</sup>	64.68 <sup>c</sup>	64.70 <sup>c</sup>	66.11 <sup>b</sup>	65.68 <sup>bc</sup>	0.444
DCPI (g/h/d)	22.46 <sup>a</sup>	21.24 <sup>b</sup>	0.106	22.25 <sup>ab</sup>	22.68 <sup>a</sup>	21.84 <sup>bc</sup>	21.08 <sup>d</sup>	21.42 <sup>cd</sup>	0.167
TDNI (g/h/d)	97.43 <sup>a</sup>	93.26 <sup>b</sup>	0.402	100.57 <sup>a</sup>	92.94 <sup>b</sup>	94.01 <sup>b</sup>	94.92 <sup>b</sup>	94.30 <sup>b</sup>	0.635
DE (Kcal/Kg)	2121.39 <sup>a</sup>	2060.10 <sup>b</sup>	10.247	2131.41 <sup>a</sup>	2056.82 <sup>b</sup>	2061.58 <sup>b</sup>	2139.84 <sup>a</sup>	2064.06 <sup>b</sup>	16.202
DEI (Kcal/h/d)	307.34 <sup>a</sup>	295.69 <sup>b</sup>	1.466	308.88 <sup>a</sup>	295.60 <sup>b</sup>	299.53 <sup>b</sup>	307.22 <sup>a</sup>	296.35 <sup>b</sup>	2.318
DEI/DCPI	13.69 <sup>b</sup>	13.95 <sup>a</sup>	0.078	13.92 <sup>b</sup>	13.04 <sup>c</sup>	13.72 <sup>b</sup>	14.59 <sup>a</sup>	13.84 <sup>b</sup>	0.124

a, b, c : Means within the same raw with different superscripts are significantly different (P < 0.05). SEM = standard error of means. \* DE (kcal/kgDM) = (5.28 × DCP) + (9.51 × DEE) + (4.20 × DCF) + (4.20 × DNFE), (Schiemann et al. (1972).

**Table 4. The interaction between feeding experimental diets without or with NaNO<sub>3</sub> and without or with feed additives on nutrient digestibility and feeding values**

Items	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	± SEM
	NaNO <sub>3</sub>			Additives							
	0.0			0.2							
	Non	Na <sub>2</sub> SO <sub>4</sub>	Clay	Yeast	Prebiotic	Non	Na <sub>2</sub> SO <sub>4</sub>	Clay	Yeast	Prebiotic	
DM intake (g/h/d)	146.23	144.67	144.69	144.44	144.35	143.6	142.7	145.89	142.67	142.76	
Nutrient digestibility (%):											
DM	72.56	70.82	68.29	70.93	68.85	70.09	71.54	69.13	69.457	68.15	0.771
OM	73.98	72.21	70.20	73.42	71.61	72.09	72.52	71.16	70.613	69.35	0.712
CP	82.18	79.97	76.33	79.03	79.01	78.29	79.63	78.77	77.213	76.02	0.631
CF	31.89	46.23	25.98	40.77	45.53	38.15	42.38	42.92	40.83	37.89	1.525
EE	89.54	57.17	54.61	73.36	79.29	77.01	81.39	74.68	65.16	80.93	3.82
NFE	80.36	80.02	82.61	82.21	78.87	81.11	80.95	79.35	79.11	78.82	0.884
NDF	40.80	44.13	38.36	47.25	39.99	48.69	40.75	38.35	40.11	34.56	3.548
ADF	45.26	34.83	27.34	34.23	28.11	28.84	31.49	30.51	36.09	30.79	5.403
Hemicellulose	58.27	59.01	57.23	67.62	59.20	62.01	55.14	51.16	47.053	40.81	4.087
Cellulose	35.02	41.01	33.89	40.87	36.59	34.71	23.85	36.44	39.227	31.26	1.665
NFC*	92.91	94.42	92.89	92.96	93.05	95.53	96.12	95.197	93.783	95.26	1.235
Digested nutrients and feeding value as DM (%):											
DCP	15.99	15.74	15.117	15.2	15.467	14.707	15.82	14.95	14.16	14.37	0.165
DCF	4.07	8.51	3.92	6.49	8.37	6.14	7.11	7.60	6.95	6.84	0.255
DEE	9.72	1.59	2.16	2.916	4.16	3.73	3.34	4.24	3.11	4.88	0.179
DNFE	42.63	39.74	42.51	42.64	39.26	41.74	37.49	38.91	40.76	38.03	0.431
TDN	72.42	65.59	63.70	67.25	67.25	66.33	63.76	65.70	64.97	64.11	0.628
DCPI (g/h/d)	23.39	22.78	21.87	21.96	22.33	21.12	22.58	21.81	20.20	20.51	0.236
TDNI (g/h/d)	105.89	94.89	92.17	97.14	97.08	95.24	90.99	95.85	92.69	91.53	0.898
DE (Kcal/Kg)	2138.40	2125.04	2050.36	2171.66	2121.46	2124.41	1988.6	2072.8	2108.02	2006.65	22.913
DEI (Kcal/h/d)	312.70	307.43	296.66	313.69	306.23	305.06	283.78	302.39	300.75	286.48	3.278
DEI/DCPI	13.37	13.50	13.57	14.29	13.72	14.47	12.57	13.87	14.89	13.96	0.175

SEM = standard error of means

The DMI g/d was higher with feeding on R<sub>1</sub> and R<sub>8</sub> than feeding on the other diets, but the DM digestibility was higher with feeding on R<sub>1</sub> and R<sub>7</sub> than the others. The OM digestibility was increased with feeding on R<sub>1</sub> and R<sub>4</sub>, CP with feeding on R<sub>1</sub>, R<sub>2</sub> and R<sub>7</sub>, CF with feeding on R<sub>2</sub>, R<sub>5</sub>, R<sub>8</sub> and R<sub>9</sub>, EE with feeding on R<sub>1</sub>, R<sub>7</sub> and R<sub>10</sub>, NFC with feeding on R<sub>3</sub> and R<sub>4</sub>, NDF with feeding on R<sub>4</sub> and R<sub>6</sub>, ADF with R<sub>1</sub> and R<sub>9</sub>, hemicellulose with R<sub>4</sub>, cellulose with R<sub>2</sub>, R<sub>4</sub>, and R<sub>9</sub> and NFC with R<sub>7</sub>, R<sub>8</sub> and R<sub>10</sub>. The DCP % was higher with feeding on R<sub>1</sub>, R<sub>2</sub> and R<sub>7</sub>, DCF with R<sub>2</sub>, R<sub>5</sub> and R<sub>8</sub>, DEE with R<sub>1</sub>,

DNFE with R<sub>1</sub>, R<sub>3</sub>, R<sub>4</sub>, R<sub>6</sub> and R<sub>9</sub>, TDN % was higher with feeding with R<sub>1</sub>, DCPI g/d with R<sub>1</sub>, R<sub>5</sub> and R<sub>7</sub>, TDNI g/d with R<sub>1</sub>, DE Kcal/Kg was higher with feeding on R<sub>4</sub> and R<sub>9</sub>, DEI Kcal/d with R<sub>1</sub> and R<sub>4</sub>, DEI/DCPI increased with feeding on R<sub>4</sub>, R<sub>6</sub> and R<sub>9</sub>.

Dietary fiber level affects the digestibility of the other nutrients in the diet and can also influence growth rate of rabbits (Gidenne and Garcia, 2006). So, the feeding on R<sub>1</sub> was lower in CF contents (12.77 %) than the other experimental diets and the NFC/NDF ratio was higher than the others. This may explain that feeding on R<sub>1</sub> caused higher DMI as well as higher digestibility of DM, OM, CP, EE, ADF, DCP and feeding values in terms of DEE, DNFE, TDN, TDNI and DEI than feeding on the other diets. The protein digestion of animal was lower with low NFC:NDF diet (Ead *et al.* 2011).

On the other hand, the requirements for fermentable N for microbial growth is optional at 30 g N/Kg organic matter digested or 15 g N/Kg DMI at 50 % digestibility (Booth and McDonald, 1982). From the presented results, it could be cleared that feeding on R<sub>9</sub> was the highest DEI/DCPI (14.89 Kcal/g DCPI) along with feeding on R<sub>6</sub> (14.47 Kcal/g DCPI) or with feeding on R<sub>4</sub> (14.29 Kcal/g DCPI) compared with the other diets. Feeding on R<sub>9</sub> increased the digestibility of CF, ADF, cellulose, DNFE and TDNI, when feeding on R<sub>6</sub> increased the digested NFE and when feeding on R<sub>4</sub> increased the digestibility of NFE, NDF, cellulose, DNFE, DE and DEI.

Tables (5 and 6) show the effect of feeding the experimental diets without or with NaNO<sub>3</sub> and without or with feed additives on caecum fermentation in terms of pH value, volatile fatty acids (VFA) (ml. eq. /100 ml) and NH<sub>3</sub> concentration (mg/100ml).

There were no significant effects with feeding diets without or with NaNO<sub>3</sub> and without or with feed additives on the pH values as shown in Table (5). The interaction among the experimental diets as shown in table (6). The pH values tend to be higher with feeding on R<sub>1</sub>, R<sub>3</sub>, R<sub>4</sub> and R<sub>9</sub> (7.64, 7.64, 7.48 and 7.33, respectively) than the other experimental diets. The pH or the concentration of the fermentation end products were not significantly affected by the feeding program. The caecal metabolism of nutrients is similar in rabbits to that can

be shown in other herbivores, but the short chain fatty acids (SCFA) pattern exhibits some differences in rabbits, namely a predominance of acetate, followed by butyrate and then by propionate (Gidenne *et al.*, 2007).

The presented data showed that the total VFA increased ( $p < 0.05$ ) with diet without NaNO<sub>3</sub> (2.85 ml. eq/100ml) than when feeding with NaNO<sub>3</sub> (2.49 ml. eq/100ml) as show in Table (5). The effect of the feed additives, also showed that the total VFA increased ( $p < 0.05$ ) without feed additives or with added Na<sub>2</sub>SO<sub>4</sub> or clay (2.85, 2.98 and 2.63 ml. eq/100ml, respectively) than added prebiotic (2.37 ml. eq/100ml), but there was no significant difference between feeding without feed additives or with added yeast or between added clay or yeast to the feeds. There was no significant difference among the experimental diets in the total VFA concentrations in the caecum as shown in table (6). The highest values were observed with feeding on R<sub>1</sub> (3.3 ml. eq/100ml), R<sub>2</sub> (3.07 ml. eq/100ml) and R<sub>7</sub> (2.90 ml. eq/100ml).

The concentration of the total VFA observed ranged from 4.3 to 8.2 mmol/100ml (Garcia *et al.*, 2002). There were no changes in the caecal VFA production in finished rabbits (Garcia *et al.*, 1995).

As shown in Table (5), there was no significant difference between the feeding diet without NaNO<sub>3</sub> (25.6 mg/100 ml) or with NaNO<sub>3</sub> (24.97 mg/100ml) on NH<sub>3</sub> concentration. The feed additives showed that NH<sub>3</sub> concentration increased ( $p < 0.05$ ) with added clay (32.53 mg/100ml) than feeding without additives or added Na<sub>2</sub>SO<sub>4</sub> or yeast or prebiotic (23.10, 25.57, 23.19 and 22.03 mg/100ml respectively). The interaction among the experimental diets on the NH<sub>3</sub> concentrations in the caecum, showed that there was no significant effect have been recorded. The highest values were observed with feeding on R<sub>3</sub> and R<sub>8</sub> diets (32.20 and 32.85 mg/100ml respectively) than the feeding on the other experimental diets. Ammonia is the main end product of N catabolism as well as the main nitrogenous source for the microbial population in the caecum (Carabano *et al.*, 1988). Ammonia is used by bacteria, in combination with carbon chain produced from carbohydrate fermentation, to synthesize new amino acids for bacteria growth (Van Soest, 1994).

**Table 5. Effect of feeding experimental diets without or with NaNO<sub>3</sub> and without or with feed additives on caecum parameters.**

Items	NaNO <sub>3</sub>		± SEM	Additives					± SEM
	0 %	0.2 %		Non	Na <sub>2</sub> SO <sub>4</sub>	Clay	Yeast	Prebiotic	
pH	7.45	7.29	0.056	7.48	7.22	7.47	7.41	7.29	0.088
VFA	2.85 <sup>a</sup>	2.49 <sup>b</sup>	0.08	2.85 <sup>ab</sup>	2.98 <sup>a</sup>	2.63 <sup>abc</sup>	2.50 <sup>bc</sup>	2.37 <sup>c</sup>	0.126
NH <sub>3</sub>	25.60	24.97	0.789	23.10 <sup>b</sup>	25.57 <sup>b</sup>	32.53 <sup>a</sup>	23.19 <sup>b</sup>	22.03 <sup>b</sup>	1.248

a, b, c : Means within the same raw with different superscripts are significantly different ( $P < 0.05$ ). SEM = standard error of means.

**Table 6. The interaction between feeding experimental diets without or with NaNO<sub>3</sub> and without or with feed additives on caecum parameters.**

Items	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	± SEM
	NaNO <sub>3</sub>			Additives							
	0.0			0.2							
	Non	Na <sub>2</sub> SO <sub>4</sub>	Clay	Yeast	Prebiotic	Non	Na <sub>2</sub> SO <sub>4</sub>	Clay	Yeast	Prebiotic	
pH	7.64	7.18	7.64	7.48	7.29	7.31	7.25	7.29	7.33	7.29	0.125
VFA	3.30	3.07	2.70	2.60	2.57	2.40	2.90	2.57	2.40	2.17	0.178
NH <sub>3</sub>	21.70	25.95	32.20	23.89	24.27	24.50	25.20	32.85	22.49	19.79	1.765

SEM = standard error of means.

Tables (7 and 8) show the data of the interactions of the effect of feeding the experimental diets on blood parameters. As showed in Table (7), there were increased ( $p < 0.05$ ) in glucose, globulin and urea - N concentrations with feeding with NaNO<sub>3</sub>, while the effect of the feed additives showed that the feeding without additives or with added yeast increased the

globulin concentration ( $p < 0.05$ ) compared with added Na<sub>2</sub>SO<sub>4</sub>, clay and prebiotic to the diets. The interaction among the experimental diets on the serum blood parameters are shown in Table (8). There was no significant effect on the blood constituents as the effect of the experimental feeding diets. The glucose concentration was higher (130 mg/100ml)

with feeding on R<sub>9</sub> than other diets. Protein concentration was higher with feeding R<sub>4</sub> or R<sub>10</sub> (6.73 and 7.00 g/100ml, respectively) than the others. Urea - N concentration was the highest with feeding on R<sub>7</sub> (60.83 mg/100ml), while the creatinine concentration was increased (2.60 mg/100ml) with feeding on R<sub>9</sub> than the other diets. The AST was increased with feeding on R<sub>1</sub>, R<sub>7</sub> and R<sub>9</sub> (53.0, 57.67 and 63 U/L, respectively) than feeding on the other experimental diets, while the ALT increased with feeding on R<sub>6</sub>, R<sub>8</sub>, R<sub>9</sub> and R<sub>10</sub> (36.0, 35.67, 37.33 and 35.67 U/L, respectively) than the other diets. The lowest value for ALT concentration was observed with feeding on R<sub>7</sub> (23.67 U/L). The AST/ALT ratio was higher (2.43) with feeding on R<sub>7</sub>, but the other diets were between 1.14 - 1.74.

(Poortmans and Dellalieux, 2000), reported that there were no significant effect changes when measuring urinary creatinine, albumin and urea in dosage range of 1.28 - 2.8 g protein/Kg body weight.

Most causes of liver cell injury are associated with greater increase in ALT than AST, however AST to ALT ratio of 2:1 or greater is suggestive of alcoholic liver disease (Moussavian et al., 1985). When the AST is higher than ALT, a muscle source of these enzymes should be considered.

The present results are in agreement with (Igwebuik et al., 2008) except for urea - N concentration which was higher with feeding on R<sub>3</sub>, R<sub>6</sub>, R<sub>7</sub>, R<sub>8</sub>, R<sub>9</sub> and R<sub>10</sub> (52.30, 51.97, 60.83, 46.5, 47.30 and 54.37 mg/100ml, respectively) than the normal range (41 - 42 mg/100ml) in rabbits (12 weeks old).

Glucose is crucial for maintenance and productive function in animals (Reynolds, 2005). In growing animal's glucose requirements will be determined by growth rate which is set by ME intake (Maklad, H. M. Eman et al, 2011) Glucose is utilized by animal cells produce energy (Richards et al., 1995). Glucose concentration was significant and positively correlated with total protein and albumin, but negatively correlated to urea. During periods of energy restrictions, the short fall in energy may be met by the catabolism of body protein. Thus more protein was metabolized to meet the energy requirements and this elevated the urea and creatinine concentration (Greenwood et al., 2002).

The ALP concentration was ranged from 69.33 to 112.0 U/L as shown in Table (19). Tork et al., (2011), found that the ALP concentration was from (51.4 - 80.64 U/100ml) in their study on growing rabbits. Serum ALP levels may increase in congestive heart failure as a result of injury to the liver (Harper et al., 1977).

**Table 7. Effect of feeding experimental diets without or with NaNO<sub>3</sub> and without or with feed additives on some blood parameters of experimental rations.**

Items	NaNO <sub>3</sub>		± SEM	Additives					± SEM
	0 %	0.2 %		Non	Na <sub>2</sub> SO <sub>4</sub>	Clay	Yeast	Prebiotic	
Glucose (mg / 100ml)	104 <sup>b</sup>	120 <sup>a</sup>	5.678	107	107	111	121	114	8.978
Total protein (g / 100ml)	5.46	6.24	0.301	6.15	5.37	5.87	6.17	5.70	0.476
Albumin (g/100ml)	4.05	4.18	0.137	4.13	4.07	3.98	4.12	4.28	0.217
Globulin (g/100ml)	1.41 <sup>b</sup>	2.06 <sup>a</sup>	0.219	2.02 <sup>a</sup>	1.30 <sup>b</sup>	1.89 <sup>b</sup>	2.05 <sup>a</sup>	1.42 <sup>b</sup>	0.346
Urea-N (mg / 100ml)	43.41 <sup>b</sup>	52.19 <sup>a</sup>	2.386	47.8	48.88	49.4	45.87	47.05	3.773
Creatinine (mg / 100ml)	0.68	1.113	0.243	0.67	0.72	0.667	1.7	0.73	0.384
AST (U / l)	43.4	52.13	4.92	47.83	46	45.67	54.83	44.5	7.78
ALT (U / l)	30.2	33.67	2.635	33.17	26.83	33	33.83	32.83	4.166
ALP (U / 100ml)	85.07	86.47	8.305	86.83	75.67	102.17	79.67	84.5	13.131
GGT (U / l)	10.13	11	2.032	12.67	10.83	8	12.5	8.83	3.213
MDA (U / l)	14.48 <sup>b</sup>	21.31 <sup>a</sup>	0.998	15.39 <sup>bc</sup>	19.62 <sup>ab</sup>	18.73 <sup>abc</sup>	21.24 <sup>a</sup>	14.51 <sup>c</sup>	1.578

a, b, c : Means within the same raw with different superscripts are significantly different (P <0.05). SEM = standard error of means.

**Table 8. The interaction among feeding experimental diets without or with NaNO<sub>3</sub> and without or with feed additives on some blood parameters of experimental rations.**

Items	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	± SEM
	NaNO <sub>3</sub>					Additives					
	0.0					0.2					
	Non	Na <sub>2</sub> SO <sub>4</sub>	Clay	Yeast	Prebiotic	Non	Na <sub>2</sub> SO <sub>4</sub>	Clay	Yeast	Prebiotic	
Glucose (mg / 100ml)	106	97	97	112	108	109	116	125	130	121	12.697
Total protein (g / 100ml)	6.03	4.37	5.77	6.73	4.40	6.27	6.37	5.97	5.60	7.00	0.674
Albumin (g/100ml)	4.03	3.83	3.77	4.50	4.13	4.23	4.30	4.20	3.73	4.43	0.307
Globulin (g/100ml)	2.00	0.54	2.00	2.23	0.27	2.04	2.07	1.77	1.87	2.57	0.490
Urea-N (mg / 100ml)	43.63	36.93	52.30	44.43	39.73	51.97	60.83	46.5	47.30	54.37	5.336
Creatinine (mg / 100ml)	0.67	0.60	0.63	0.80	0.70	0.67	0.83	0.70	2.60	0.77	0.544
AST (U / l)	53.00	34.33	38.67	46.67	44.33	42.67	57.67	52.67	63.00	44.67	11.003
ALT (U / l)	30.33	30.00	30.33	30.33	30.00	36.00	23.67	35.67	37.33	35.67	5.892
ALP (U / 100ml)	96.67	68.00	112.00	69.33	79.33	77.00	83.33	92.33	90.00	89.67	18.57
GGT (U / l)	10.33	11.33	6.67	16.00	6.33	15.00	10.33	9.33	9.00	11.33	4.544
MDA (U / l)	12.31	13.30	14.52	23.70	8.58	18.46	25.93	22.94	18.78	20.44	2.231

SEM = standard error of means.

This study revealed that the impaired observed in rabbits fed the highest levels of fiber might be explained by higher fermentation losses in caecum together with an insufficient glucose from the gut to meet the requirements, while the importance of the amino acids depends on the efficiency of microbial protein synthesis. These observations are in agreement with the present results of fiber and CP

digestibility of the experimental diets and on the TDNI and DEI.

The DCPI g/d was higher with feeding on R<sub>1</sub>, R<sub>5</sub> and R<sub>7</sub>, TDNI g/d was higher with R<sub>1</sub>, DE Kcal/Kg was higher with feeding on R<sub>4</sub> and R<sub>9</sub>, DEI Kcal/d with R<sub>1</sub> and R<sub>4</sub>, DEI/DCPI increased with feeding on R<sub>4</sub>, R<sub>6</sub> and R<sub>9</sub>.

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