Evaluation of various salt addition levels in the pellets on performance and health of the domestic rabbit urination system

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Abstract. This research aims to evaluate the various salt addition levels in the pellets on the performance and health of the domestic rabbit urination system. The completely randomized design method was used to determine the 5 levels of salt addition to the treatment, namely P1, P2, P3, P4, and P5 with 0%, 0.175%, 0.25%, 0.375%, and 0.50%. The sample consists of 20 male domestic rabbits aged 8 weeks with an average body weight of 897.05 ± 68.27g, which were kept in individual cages. The variables of these rabbits were analyzed to determine their performance and urinalysis using ANOVA (analysis of variance) and correlation analysis. The ANOVA results showed that salt addition levels in the pellets had a significant effect on FI, WI, FCR, UV, Nit, Pro (p<0.05), and insignificant on BWG, pH, Leu, Uro, Blo, Ket, Bil, Glu, and SG (p>0.05). The results further showed the correlation on several variables of performance and urinalysis. In conclusion, the optimal level of salt addition in the pellets was 0.25% because it gave the lowest value of feed conversion ratio and does not adversely affect the health of the domestic rabbit urination system.

1. Introduction

Sodium (Na) and chloride (Cl) are the essential minerals needed by rabbits for healthy living. Sodium and chloride function as cation and anion by keeping the ionic and osmotic balance of intracellular and extracellular fluid, thereby triggering the electrical potential of body tissues, activation, and transmission of nerve impulses [1]. Chloride also plays an essential role in body fluid regulation, maintaining electrolytes balance, electrical neutrality, and acid-base status [2]. Both sodium and chloride are needed for rabbit growth, maintenance, gestation, and lactation in percentages of 0.2% and 0.3% [3]. Generally, forages are deficient in sodium [4],[5] because it is not an essential mineral [6]. Therefore, to prevent rabbits from becoming deficient in sodium, they need to consume sodium-deficient forages in large quantities along with the addition of salt in the pellets [3],[7].

According to [8], salt consists of sodium and chloride (NaCl), which are usually absorbed in the small and large intestine and excreted by the kidneys through urine [2],[9],[10]. The urination system consists of kidneys, ureters, bladder, and urethra that function to produce, collect, transport, store, and excrete urine containing end-body metabolite products and toxic wastes [11]. An increase in the salt level in pellets has the ability to adversely affect the health of domestic rabbits [12]. Therefore, this research aims to evaluate the various salt levels addition in the pellets on performance and health of the domestic rabbit urination system. Finally, the optimal level of salt addition in the pellets can be
determined to improve performance and not adversely affect the health of the domestic rabbit urination system.

2. Material and methods

2.1. Animals and feed

The sample consists of 20 male domestic rabbits aged 8 weeks with an average body weight of 897.05±68.27 g kept in individual cages. Feed formulation was adapted to determine the nutrient requirements of growth rabbits based on SNI 8509:2018 [13]. Furthermore, the pellet nutrients values such as dry matter, crude fiber, and salt (NaCl) were analyzed based on the method of SNI 01-2891-1992 [14]. Meanwhile, the ash, crude protein, crude fat, and calcium (Ca) were analyzed based on AOAC 2005 [15]. Finally, the nitrogen-free extract (NFE), phosphor (P), and gross energy were analyzed based on differentiation, gravimetry, and bombcalorimeter methods. The formulation and nutrients value of the experimental pellets are shown in Table 1.

| Feedstuffs         | Formula of the research pellet (%) |
|--------------------|-----------------------------------|
|                    | P₁  | P₂  | P₃  | P₄  | P₅  |
| Corn meal         | 18,94 | 18,89 | 18,83 | 18,78 | 18,73 |
| Soybean meal      | 23,05 | 23,07 | 23,08 | 23,10 | 23,12 |
| Palm kernel meal  | 16,70 | 16,69 | 16,68 | 16,67 | 16,66 |
| Rice bran         | 15,01 | 14,99 | 14,97 | 14,95 | 14,94 |
| Field grass       | 20,98 | 21,00 | 21,02 | 21,04 | 21,05 |
| Crude palm oil (CPO) | 4,62 | 4,535 | 4,47  | 4,385  | 4,30  |
| CaCO₃             | 0,40  | 0,40  | 0,40  | 0,40  | 0,40  |
| Premix            | 0,30  | 0,30  | 0,30  | 0,30  | 0,30  |
| Salt (NaCl)       | 0,000 | 0,125 | 0,250 | 0,375 | 0,500 |
| Total             | 100,00 | 100,00 | 100,00 | 100,00 | 100,00 |

Table 1. Formulation and nutrients value of the experimental pellets.

Nutrients value

|                       | P₁  | P₂  | P₃  | P₄  | P₅  |
|-----------------------|-----|-----|-----|-----|-----|
| Dry matter (%)        | 92,08| 90,97| 92,48| 91,27| 91,38|
| Ash (%)               | 6,75 | 6,44 | 6,78 | 6,74 | 7,24 |
| Crude fat (%)         | 5,16 | 5,30 | 5,45 | 5,51 | 5,86 |
| Crude fiber (%)       | 17,60 | 16,25 | 16,13 | 15,89 | 13,99 |
| Crude protein (%)     | 18,32 | 17,55 | 20,23 | 20,46 | 20,65 |
| Gross energy (cal/g)  | 4768 | 4643 | 4456 | 4468 | 4480 |
| Ca (%)                | 0,51 | 0,45 | 0,54 | 0,44 | 0,44 |
| P (%)                 | 0,80 | 0,96 | 0,82 | 0,86 | 0,92 |
| NFE (%)               | 44,25 | 45,43 | 43,89 | 42,67 | 43,64 |
| NaCl (%)              | 0,37 | 0,69 | 0,76 | 1,01 | 1,28 |

*Results of analysis of pellet nutrients value at the Laboratory of Feed Science and Technology, Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University (2020).

2.2. Experimental design

This research used the completely randomized design method with the addition of P₁, P₂, P₃, P₄, and P₅ pellets with 0%, 0.175%, 0.25%, 0.375%, and 0.50% levels of salt. The analyzed variables were performance (feed intake (FI), water intake (WI), body weight gain (BWG), feed conversion ratio (FCR)) and urinalysis (urine volume (UV), urine pH (pH), leukocyte (Leu), nitrite (Nit), urobilinogen (Uro), protein (Pro), blood (Blo), ketone (Ket), bilirubin (Bil), glucose (Glu), and specific gravity (SG)). Feed and water intakes were counted daily to determine the amount of used and remaining pellets. The body weight gain was measured weekly by subtracting the initial value from the final.
feed conversion ratio was calculated weekly by dividing the feed intake by body weight gain. Urine volume and pH values were measured daily using a measuring cylinder and pH meter. Meanwhile, leukocyte, nitrite, urobilinogen, protein, blood, ketone, bilirubin, glucose, and urine specific gravity were qualitative-analyzed weekly using reagent stripe for urinalysis (dipstick) URS-10T. This process was also carried out by matching the color change of the reagent stripe to the charts and reading time on the bottle label.

2.3. Data analysis

The data obtained were analyzed to determine their variance (Anova) and correlation. The post-hoc test by Duncan means range test (DMRT) is performed assuming the results of Anova are significantly different at significance level 0.05.

3. Results and discussion

3.1. Rabbit performance

The results of feed intake, water intake, body weight gain, and feed conversion ratio as the performance variables are shown in Table 2. The Anova values show that there were significant differences in feed intake, water intake, and feed conversion ratio (p<0.05) and insignificant differences in body weight gain (p>0.05).

Table 2. Rabbit performance.

| Treatments | Performance variables |
|------------|------------------------|
|            | FI (g/day) | WI (ml/day) | BWG (g/week) | FCR |
| P1         | 63.82 ± 15.10<sup>a</sup> | 162.39 ± 74.06<sup>b</sup> | 77.31 ± 27.52 | 6.13 ± 1.59<sup>ab</sup> |
| P2         | 62.46 ± 12.33<sup>a</sup> | 122.09 ± 36.82<sup>a</sup> | 83.19 ± 15.66 | 5.34 ± 0.71<sup>ab</sup> |
| P3         | 67.97 ± 13.32<sup>a</sup> | 148.73 ± 54.77<sup>ab</sup> | 97.94 ± 11.68 | 4.90 ± 0.86<sup>a</sup> |
| P4         | 113.74 ± 13.25<sup>c</sup> | 210.35 ± 33.52<sup>c</sup> | 96.50 ± 28.02 | 8.63 ± 1.76<sup>c</sup> |
| P5         | 95.31 ± 15.88<sup>b</sup> | 137.30 ± 28.16<sup>ab</sup> | 97.17 ± 17.73 | 6.97 ± 1.09<sup>bc</sup> |

P<sub>1</sub>: pellets with no salt, P<sub>2</sub>: pellets with 0.125% salt, P<sub>3</sub>: pellets with 0.250% salt, P<sub>4</sub>: pellets with 0.375% salt, P<sub>5</sub>: pellets with 0.500% salt, FI: feed intake, WI: water intake, BWG: body weight gain, FCR: feed conversion ratio. Numbers followed with a different superscript in the same column showed the significantly different based on DMRT with significance level 0.05.

3.1.1. Feed intake. The DMRT result showed that pellet with 0.375% salt (P<sub>4</sub>) is the treatment with the highest feed intake. This is presumably because the rabbits in P<sub>4</sub> have sodium appetite, which was a strong motivation to consume a large level of salt due to sodium deficiency [16]. Furthermore, the addition of salt enhanced the food savour and encouraged its high consumption [17],[18],[19]. In this research, the highest level of salt addition was in P<sub>5</sub> with 0.500% in the pellets, while the most significant feed intake was not in that treatment. This was presumably because the high level of salt addition limits and rejects the feed factor [12]. The high level of salt also has the ability to trigger the activation of sour and bitter taste bud cells, which leads to feeding rejection [20].

3.1.2. Water intake. The DMRT result showed that pellets with 0.375% salt (P<sub>4</sub>) are the treatment with the highest water intake, while pellets with 0.125% salt (P<sub>2</sub>) are the lowest. The highest feed intake is in the P<sub>4</sub> with a salt addition level of 0.375%. The high water intake in P<sub>4</sub>, presumably according to the rabbit physiological response to anticipated rise in feed intake, did not lead to hyperosmolality capable of affecting the excessive increase of plasma volume (hypervolemia) and hypertension. Furthermore, the balance-body fluid regulation (osmoregulation) occurred in this pellet, while the osmoreceptor in the brain section organum vasculorum lamina terminalis (OVLT) stimulated thirst by activated the median preoptic (MnPo) area [21],[22]. Therefore, the rabbit increased the water intake in order to balance the plasma volume.
3.1.3. Body weight gain. There was no significant difference in body weight gain based on Anova (p>0.05). Studies carried out by [23] and [24], Anova on rabbit body weight gain showed insignificant differences. Average rabbits’ body weight gain in this research was 77.31-97.94 g/week or approximately 11.04-13.99 g/day, which is still in accordance with those in the tropics, in the range 10-20 g/day [25]. Based on the result of this research, it can be stated that salt levels addition had no significant effect on the body weight gain even though the feed intake is significantly different.

3.1.4. Feed conversion ratio. The DMRT result showed that the lowest and highest values of feed conversion ratio were in the P3 and P4. This means that the pellets’ P3 and P4 with 0.25% and 0.375% salt addition were the most efficient and ineffective conversion as body weight gain, respectively.

3.2. Urinalysis

The urinalysis results are shown in Table 3. It indicates that there were significant differences in urine volume, nitrite, and protein (p<0.05), while on urine pH, leukocyte, urobilinogen, blood, ketone, bilirubin, glucose, and specific gravity were insignificant (p>0.05).

Table 3. Rabbit urinalysis result.

| Treatments   | P1                | P2                | P3                | P4                | P5                |
|--------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| UV (ml/day)  | 76.73±44.81<sup>a</sup> | 45.75±15.12<sup>a</sup> | 63.05±37.90<sup>b</sup> | 74.40±22.93<sup>b</sup> | 38.12±23.86<sup>a</sup> |
| pH           | 8.3 ± 0.1         | 8.2 ± 0.2         | 8.3 ± 0.2         | 8.2 ± 0.1         | 8.3 ± 0.2         |
| Leu (cacells/µL) | Negative          | Negative          | Negative          | Negative          | Negative          |
| Nit (-/-)    | Negative<sup>a</sup> | Negative<sup>a</sup> | Negative<sup>b</sup> | Negative<sup>a</sup> | Negative<sup>b</sup> |
| Uro (µmol/L) | Normal            | Normal            | Normal            | Normal            | Normal            |
| (3,2-16)     | (3,2-16)          | (3,2-16)          | (3,2-16)          | (3,2-16)          |                  |
| Pro (g/L)    | ++++ (≥20)<sup>a</sup> | ++++ (≥20)<sup>ab</sup> | ++++ (≥20)<sup>bc</sup> | ++++ (≥20)<sup>abc</sup> | ++++ (≥20)<sup>c</sup> |
| Blo (cacells/µL) | Negative          | Negative          | Negative          | Negative          | Negative          |
| SG           | 1,000             | 1,000             | 1,000             | 1,000             | 1,000             |
| Ket (mmol/L) | Negative          | Negative          | Negative          | Negative          | Negative          |
| Bil (µmol/L) | Small (17)       | Small (17)       | Small (17)       | Small (17)       | Small (17)       |
| Glu (mmol/L) | Negative          | Negative          | Trace (5)         | Trace (5)         |                  |

P<sub>1</sub>: pellets with no salt, P<sub>2</sub>: pellets with 0.125% salt, P<sub>3</sub>: pellets with 0.250% salt, P<sub>4</sub>: pellets with 0.375% salt, P<sub>5</sub>: pellets with 0.500% salt, UV: urine volume, pH: urine pH, Leu: leukocyte, Nit: Nitrite, Uro: Urobilinogen, Pro: protein, Blo: blood, SG: specific gravity, Ket: ketone, Bil: bilirubin, Glu: glucose.

Numbers followed with a different superscript in the same row showed significantly different based on DMRT with significance level 0.05.

The DMRT results showed that pellets with 0% (P<sub>1</sub>), 0.250% (P<sub>2</sub>), and 0.375% (P<sub>4</sub>) salt were the treatment with the highest urine volume, while those with 0.125% (P<sub>3</sub>) and 0.500% (P<sub>5</sub>) were the lowest. Urine volume in this research was approximately 38.12-76.73 ml/day and included in the normal category in the range of 20-350 ml/kg of body weight [26]. Overall, nitrite was negative, but in time of research and analysis, the most frequently positive nitrite was in P<sub>3</sub> and P<sub>5</sub> than in P<sub>1</sub>, P<sub>2</sub>, P<sub>4</sub>. Furthermore, the urine contained in nitrite is called nitrituria, and it is a sign of urinary tract infection when it occurs [27],[28]. Protein with ≥20 g/L urine was mostly detected in P<sub>5</sub>, while in P<sub>1</sub> was rarely detected. The urine contains a protein called proteinuria, and the possible causes were glomerular leakage and urinary tract infection [26]. Glomerular leakage made the protein filtration inefficient, therefore, it flows freely into the urine. Friction between the urinary tract and salt crystals is presumably the cause of glomerular leakage and urinary tract infection. However, the urine protein content of up to 20 mg/dl is still considered normal [26].
The urine pH in this research was approximately 8.2-8.3, and still normal in the range of 8.2-9 [29]. Rabbit urine specific gravity in all treatments was 1.00 and below the normal value of 1.003-1.036 [26],[29]. The low value of this urine specific gravity presumably is due to the increase in urine production (diuresis osmotic), medullary disorders, resistance, and deficient antidiuretic hormone [26]. Based on this research, the consumption of pellets with salt addition increased water intake and urine production, which was one of the causes of the low value of its specific gravity. The use of a refractometer in the measurement was also recommended to get more valid results [30].

There was no glucose detected in urine P1, P2, and P3, while in P4 and P5, trace glucose was found (5 mmol/L urin). The presence of glucose in urine, called glucosuria, occurred during hyperglycemia and exceeded the kidney filtration threshold, decreasing kidney tubules reabsorption ability [30] to enable the free flow of glucose in urine. However, to ensure this, blood glucose needs to be checked [31].

Urobilinogen and bilirubin contents were normal (3.2-16 µmol/L urin), and trace (17 µmol/L urin), while leukocyte, blood, and keton were not detected in the rabbit urine. Based on this urinalysis, it is known that rabbit urine characteristics for each treatment were normal. Therefore, the pellets’ salt addition up to 0.500% did not adversely affect the domestic rabbit urination system.

3.3. Correlation of variables
The correlation of variables from 0.00-0.10; 0.10-0.39; 0.40-0.69; 0.70-0.89; and 0.90-1.00 are in the negligible, weak, moderate, and very strong correlation [32]. Sign of correlation coefficient shows the negative direction means that the variables are inversely related, and vice versa [33].

Table 3 shows that the feed intake had a positive-moderate correlation with water (0.65), body weight gain (0.49), and feed conversion ratio (0.58). This means that an increase in the feed intake value is in line with a rise in water intake, body weight gain, and feed conversion ratio. Water intake had a positive-weak correlation with feed conversion ratio (0.48) and a positive-strong correlation with urine volume (0.72). Therefore, the greater the amount of water consumed by rabbits, the higher the feed conversion ratio value and the more the urine excreted. Furthermore, urine volume had a negative-strong correlation with the pH (-0.72) and a negative-moderate correlation with nitrite (-0.47). The increased urine volume decreases the pH and nitrite values, and vice versa. Urine pH had positive-strong correlation with nitrite (0.72) and positive-moderate correlation with glucose (0.45). The increase in pH value in line led to a rise in urine nitrite and glucose values. Glucose also had a positive-moderate correlation with body weight gain (0.55), which means that the higher the value, the greater the glucose in the urine. Lastly, protein had a negative-moderate correlation with blood (-0.60), which means an increase in urine protein value led to a decrease in urine blood content and vice versa.
Table 4. The correlation coefficients of variables.

|       | FI   | WI   | BWG  | FCR  | UV   | pH   | Leu  | Nit  | Uro  | Pro  | Blo  | SG   | Ket  | Bil  | Glu  |
|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| FI    | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| WI    | 1.00 | 0.65*|      |      |      |      |      |      |      |      |      |      |      |      |      |
| BWG   | 1.00 | -0.39| 0.48*|      |      |      |      |      |      |      |      |      |      |      |      |
| FCR   | 1.00 | 0.36 | -0.21| 0.14 |      |      |      |      |      |      |      |      |      |      |      |
| UV    | 1.00 | -0.72*| -0.47*| -0.15| -0.16|      |      |      |      |      |      |      |      |      |      |
| pH    | 1.00 | 0.39 | 0.23 | 0.26 | 0.32 | 0.15 |      |      |      |      |      |      |      |      |      |
| Leu   | 1.00 | 0.32 | 0.16 | 0.06 | 0.15 | 0.15 | 0.26 |      |      |      |      |      |      |      |      |
| Nit   | 1.00 | -0.39| -0.32| -0.10| -0.05| 0.17 | 0.06 | 0.25 |      |      |      |      |      |      |      |
| Uro   | 1.00 | 0.50*| -0.17| -0.16| 0.11 | 0.17 | 0.45*| 0.04 | 0.25 |      |      |      |      |      |      |
| Pro   | 1.00 | 0.50*| -0.32| -0.10| -0.13| -0.55*|      |      |      |      |      |      |      |      |      |
| Blo   | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| SG    | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Ket   | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Bil   | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
| Glu   | 1.00 |      |      |      |      |      |      |      |      |      |      |      |      |      |      |

** : correlation is significant at the 0.01 level (2-tailed)
* : correlation is significant at the 0.05 level (2-tailed)
c : cannot be computed because at least one of the variables is constant
4. Conclusion
In conclusion, the optimal level of salt addition in the pellets was 0.25% because it gave the lowest value of feed conversion ratio and did not adversely affect the health of the domestic rabbit urination system.

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