The effect of dietary forages in tetanus hyper immunized equine serum production

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Abstract. The determination of local forage in meeting the nutritional requirement of horses and its effect on the antibody titer production is necessary to be conducted. The aim of the study was to determine the effect of dietary forage to antibody titer production of Anti-Tetanus Serum. All procedures performed in this study were approved by the Institutional Animal Care and Use Committee (Ethical Approval No: 01/IACUC-BF/VI/20). A total of 12 Sandalwood horses aged 3-6 years with body weight ranged at 268.4 - 365.8 kg were used in this study. Horses were randomly assigned into four dietary treatment groups for ten weeks (100% Elephant grass; 100% Mott elephant grass; 67% Elephant grass: 33% Kikuyu grass; and 67% Mott elephant grass: 33% Kikuyu grass) which allowed 7.24; 11.45; 11.12; 12.62% crude protein respectively. The titer measurement was carried out once a week during the production period. The data obtained was analyzed using correlation analysis. The results showed that 100% Mott elephant grass group produced the highest titer but the correlation between crude protein levels in the diets and titer were weak (0.44). Thus, it can be concluded that the high protein forage given has no potential to increase the serum antibody titer.

1. Introduction
Tetanus is an acute infectious disease caused by tetanospasmin and tetanolysin toxins from the gram-positive bacteria Clostridium tetani. Clostridium tetani spores contaminate soil, debris, and animal feces [1]. Infection can occur when bacteria enter the human body through wounds and the neurotoxin circulates through the blood vessels and is distributed to peripheral areas and central nervous system (CNS) resulting in tetanic muscular spasms, dysphagia, and autonomic dysfunction [2]. In developing countries it is reported that the death rate due to tetanus is 10–50%. In Indonesia, tetanus is the most common cause of death in neonates and after natural disasters where access to hospital for appropriate treatment is limited. Tetanus is still a health problem in the world. Efforts that can be made to prevent tetanus can be done with complete TT immunization in pregnant women and locations affected by natural disasters [3]. The tetanus vaccine used in Indonesia is a type of DT and TT produced by PT Biofarma. In the production process of the TT vaccine, horses are used as hyper immunized animals because horses are the most susceptible of all domestic animals [4].
The horse belongs to the group of mono gastric herbivores adapted to eating plant-fiber or forage-based diets [5]. Its specialized gastrointestinal tract (GIT) is able to ferment varied species of plant [6] with the help of microbe population in an organ known as caecum to break down the plant’s cell wall called cellulose [7]. The performance produced by horses will go parallel with the forage quality which good quality forage will make a great horse performance. Good forage, of course, not only as a source of energy, but also as a source of protein, vitamins, minerals and nutrients [8]. Therefore, grass and hay were used as a major component of the horse’s diet. However, forage frequently will not provide all the macro and micronutrients required, even for horse maintenance, so the diet should be completed by other nutrients from feed pellet concentrate and supplement. Most common subtropics grass fed to horses includes Bahia (Paspalum notatum Flugge), Bermuda (Cyonodon dactyon (L) Pers.), Digitaria (Digitaria decumbens Stent), Ryegrass (Lolium perenne L.), Pearlmillet (Pennisetum americanum (L). Leeke) [9]. According to Parakassi [10], in Indonesia and other tropical regions there is no clear information regarding the existence of forage that stands out for its quality, especially for horse’s feed. Consequently horse owners provide imported feed to meet the nutritional needs of the horse. To get the best horse performance needs evaluation and determination of the quality of horse forage. Moreover, there was a study that the protein digestibility of imported feed was significantly higher than that of local feed protein [11]. Therefore it is necessary to conduct research related to local horse feed in an effort to determine the types of local forage in meeting the nutritional needs of horses and their effect on the antibody titer produced so that the horse is expected to perform well according to its utility. With this information, it is hoped that it can reduce importation of horse feed and develop high protein local forage cultivation based on tetanus antisera titer production. The hypothesis tested that giving different dietary forage that contains different percentages of crude protein may affect antibody titer production.

2. Materials and methods

2.1. Animals, diets, and experimental design
All procedures performed in this study were approved by the Institutional Animal Care and Use Committee (IACUC), PT Biofarma (PERSERO) (Ethical Approval No: 01/IACUC-BF/VI/20). A total of 12 Sandelwood horse aged 3–6 years with body weight (BW) 300-350 kg were used in this study. Horse were randomly assigned into four groups and fed diets with four different forage ratio (100% elephant grass, 100% mott elephant grass, 67% Elephant grass: 33% Kikuyu grass, and 67% mott elephant grass: 33% Kikuyu grass; namely groups 1, 2, 3, and 4, respectively as the sole forage. Concentrate pellet was given 2.61kg/day for each animal. Water was available ad libitum. Group 2 and 4 were fed with an adaptation diet containing 50% existing forage (Elephant grass) and 50% new forage (mott Elephant grass) for 3 weeks, and then transferred to treatment diets for 10 weeks that were formulated. Horses were housed in individual stalls in a ventilated, environmentally controlled barn with wood shaved bedding.

2.2. Blood sampling
Twenty ml blood samples were taken before and after tetanus immunization of the horse according to individual schedules. These samples were taken from the jugular vein via catheters and placed in non EDTA sampling tubes. The blood samples were stored on ice until centrifugation. The samples were centrifuged at 3,000 rpm for 3 minutes. Serum was pipetted to a tube and stored at a temperature of -20°C until the analysis.

2.3. Tetanus neutralizing antibody titer
The antibody titer was measured once a week on horses that are in production period. The horse that produces less antibody titer of the standard will rest. Testing for antibody titer against anti-tetanus serum (ATS) was performed using the in vitro Ramon flocculation test. Process of these tests is to make a dilute series of antigen added with serum with the same volume for each tube then incubated in in a
water bath with a temperature of 45°C until there is a cloud like mass on the surface (has the occurrence of flocculation). Flocculation occurs when antigens and antibodies are present in equilibrium. The formula for calculating the titer value (lf / ml) of tetanus / diphtheria antibody is as follows:

$$Lf/ml = \frac{\text{Standard volume of Toxoid (ml)} \times [\text{raw Toxoid}] \times \text{dilution factor}}{\text{Volume of material test (ml)}}$$

The horse is declared to pass the titer check when the horse is up for production ATS has a titer with a minimum limit of 500 lf / ml [12].

2.4. Statistical analysis

The data was obtained and analyzed using Pearson’s correlation analysis. The relationship could be seen from the magnitude of the correlation value where 0.7 indicates a significant and positive relationship exists between two variables [13].

3. Results and discussion

In this experiment, proximate composition of forages was analyzed by Methodology AOAC 2012 as shown in Table 1.

| Forages          | Dry Matter (%) | Crude Protein (%) | Crude Fiber (%) | Crude Lipid (%) | NFE (%) | Gross Energy (Kcal/kg) | Digestibility (%) |
|------------------|----------------|-------------------|-----------------|-----------------|---------|------------------------|------------------|
| Gajah            | 29.60          | 7.24              | 36.89           | 1.64            | 42.48   | 35.11                  | 56.95            |
| Odot             | 31.82          | 11.45             | 15.23           | 26.34           | 3.10    | 38.03                  | 58.25            |
| Gajah + Kikuyu   | 36.25          | 9.80              | 9.80            | 34.12           | 1.75    | 44.00                  | 61.60            |
| Odot + Kikuyu    | 37.36          | 12.62             | 13.44           | 28.91           | 2.65    | 40.00                  | 59.36            |

Proximate analysis was conducted in Nutrition Laboratory of LAPTIAB Centre for Agriculture and Production Technology, Agency for The Assessment and Application Technology.

Crude protein is commonly used to estimate protein content whereas amino acid is a better measure of protein quality [14]. Horses can produce amino acid itself called non-essential amino acid. Essential amino acids must be provided in the horse diet since the horse can synthesize them in quantities to meet the horse's requirement. Essential amino acids Lysine, Methionine, and Threonine are the best indicators listed on commercial feed tags [15].

![Figure 1](image.png)

Figure 1. Antibody titer level (a); Crude protein consumption (b) (kg/day) in hyper immunized horse receiving different type of dietary forage

As shown in Figure 1(a), the highest titer was achieved by Odot dietary treatment. The median titer for the Elephant grass, Elephant&Kikuyu, and Odot&Kikuyu feed groups was at the interval 0-500lf/ml, while the median for the Odot group was at intervals of 500–100lf/ml. From the distribution above, it can be seen that the Odot group has a very high value above the normal diversity. This can be
seen from the picture of the wide box. The minimum titer was recorded at 600lf/ml and the maximum at 1,500lf/ml. There was a horse that did not respond to the vaccination (titer value was 0 lf/ml) is still unclear [16]. The possible cause was affinity maturation of individual antibodies. This kind of horse usually needs two doses of antigen administration [17].

As shown in Figure 1 (b) Crude protein consumption for Gajah group was at interval 0.14–0.16 kg/day, for Odot group was at interval 0.12–0.14 kg/day and the rest group was at 0.18–0.20 kg/day because Gajah&Kikuyu and Odot&Kikuyu are more digestible than Gajah and Odot grass. It has similar results with Harris et al. [5] the feed intake and interactions may be influenced by the nutrient content and digestibility of horse’s forage.

Result (Figure 2) shows that crude protein consumption has no correlation to titer measurement. This was similar with the previous research [18] that high dietary protein concentration and quality had a minor impact on immune function of healthy adult cats. Moreover, Tang et al. [19] stated that dietary protein intake had an interaction effect on animal’s breed. Landrace pigs have more benefit of high dietary protein intake than Bama-mini pigs for their immune status. Experiments of interaction between crude protein and immunity are mostly carried out on chickens to formulate new feed. Fosoul et al. [20] and Kamely et al. [21] found that no relationship between dietary crude protein level and antibody production even reduced crude protein diets may enhance the capacity of broilers to recruit leukocytes upon infection.

This study result also showed that the content of crude protein in all dietary treatments have met the needs of basal energy requirement, so it has no effect on the serum antibody production. Studies have shown that dietary protein may indirectly turnover serum protein, globulin or gamma globulin in particular. Dietary protein which may be the most suitable for growth, reproduction or maintenance of nitrogen equilibrium may not necessarily support immune response [22].

4. Conclusion
Giving different dietary forage to hyper immunized horses has no potential to increase serum antibody titer production. The highest antibody titer up to 1500lf/ml was found in the Odot group.

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