The digestive and physiological visceral organs of male Bali cattle were fed with cocoa bean shell

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Abstract. Cocoa bean shell (CBS) is a waste from cocoa bean processing, and recently used as supplements in ruminants feed. However, a CBS contains theobromines that well known for its anti-nutrient effect in the animal digestive system. This study examined the effect of CBS supplementation on some digestive organs in Bali cattle bulls. The total of 12 Bali bulls (average initial body weight, 159.25 ± 15.68 kg) aged 16 months age were fed with four levels of CBS concentrate in the diet for 16 weeks (0, 3, 6, and 9%, respectively). At the end of the experiment, digestive tract and some visceral organs weight (e.g., rumen, digestive tracts, lymph, liver, lungs, heart, and kidney) were measured and calculated as the percentage of body weight (BW). The results showed that the percentage of spleen, liver and kidney weights were increased (p<0.05) according to the level of CBS supplementation. The effect of CBS feeding on the percentage of spleen weight was occurred at level 9%, and significantly higher (p<0.05) compared to the level of 3%, 6% and without cocoa shell. The liver and kidney percentages were higher (p<0.05) at levels 6% and 9% compared to 0% and 3%. There was no effect of supplementation on the digestive tract, lungs and heart. The study concluded that CBS supplementation at 6% and 9% levels had an effect on physiological visceral organs of Bali cattle bulls.

1. Introduction
Increasing meat production can be done through an increasing population and production efficiency of livestock. Using feed from agricultural and industrial waste is an alternative to increase environmentally friendly livestock production. Cocoa bean shell (CBS) is one of the agricultural industries wastes were potentially to use as feed supplements for livestock. The potential of CBS in Indonesia is very large. BPS data for 2017 shows that cocoa production in 2017 reached 658 tons [1], those an estimated production of CBS of 112 tons per year. CBS contains 68.8% dry matter, 6.8% fat, 25.1% fiber and 16.7% crude protein. It is also has a high palatability for cattle with total digestible nutrient (TDN) is equivalent to grass, 52.3% [2], CBS also contains other beneficial polyphenol compounds as in cocoa, especially in the form of catechins, flavonol glycosides, anthocyanins and procianidin [3], theobromine is the main responsible factor for cocoa’s effects on body weight gain as well as on lipid and glucose metabolism [4], event they also had potency to be an alternative antibiotic for animal farm [5].

However, there are weaknesses in the content of anti-nutrition substances such as tannins and several types of alkaloids including theobromin. In our previous study [6], we found that high concentration of theobromine effected on nitrogen urea in blood plasma, in other hand in limited use, the presence of anti-nutrients can help in body metabolism as an antioxidant. Theobromine and some phlavonoids work
in energy metabolism, including the process of forming, storing and utilizing muscle fat and glycogen. However, the study on CBS as feed supplementation, and its depth effects on digestive and metabolism are needed. This study aimed to measure the effect of providing various levels of CBS (as supplement) on the development of some visceral organs of Bali cattle after fattening for 4 months. The research is expected to find the right formulation of CBS as supplementation for Bali cattle in forages basal feed.

2. Research method
The study was conducted in cattle fattening pen, CV Akbar Jaya, Makassar. The study used 12 1.5-year-old bulls with an average body weight of 159.25 ± 15.68 kg, body condition score was 2 on a scale of 5. Slaughtering of cows was done after 12 hours fasting, carried out at Tamangapa Animal Slaughterhouse, Antang-Makassar.

The study was arranged according to a completely randomized design with 4 treatments of CBS supplemented concentrate, A = 0%; B = 3%; C = 6%; and D = 9%. Each treatment was repeated three times. Further tests for multiple comparisons use the Tukey-HSD test. The administration of worming was carried out during the feed adaptation period in the initial 2 weeks of the study. Weighing was done every 14 days, for 3 months. Measurement of the final weight was done prior the cattle were slaughtered; the end of fasting for 12 hours. Measurement of the weight of digestive organs and visceral organs was carried out after slaughtering cattle at the end of the study. The parameters measured include body weight, rumen, intestine, liver, heart, lungs, kidneys, and spleen. The percentage of organ weight was obtained from the distribution of organ weight to cow’s body weight before slaughter.

3. Result and discussion
The analysis showed an increase (p<0.05) in the percentage of spleen, liver, and kidney weights, while the digestive tract, rumen, lungs and heart showed no significant differences. The complete results of the study are presented in Table 1.

| Visceral organs       | Cocoa beans shell levels | SE  | P     |
|-----------------------|--------------------------|-----|-------|
|                       | 0            | 3   | 6    | 9    |
| Rumen                 | 3.06         | 2.98| 2.97 | 2.95 | 0.057| 0.086|
| Digestive tract       | 1.71         | 1.69| 1.69 | 1.66 | 0.023| 0.109|
| Spleen                | 0.53         | 0.55| 0.58 | ab  | 0.68 | b    | 0.055| 0.037|
| Liver                 | 2.71         | 2.86| 2.89 | b   | 3.02 | b    | 0.136| 0.018|
| Lungs                 | 0.86         | 0.90| 0.86 | 0.90 | 0.037| 0.462|
| Heart                 | 0.79         | 0.82| 0.82 | 0.86 | 0.040| 0.126|
| Kidney                | 0.35         | 0.38| 0.43 | bc  | 0.47 | c    | 0.056| 0.011|

Least squares means within a row with a common superscript letter differ (P<0.05)

Analysis of variance showed that there was no difference in rumen percentage due to treatment. This indicated that a CBS increase of up to 9% in feed has no direct effect on rumen structure or on the rumen epithelial layer. However, there was a decrease in rumen weight percentage in line with the CBS level increase. Likewise, in the digestive organs, lungs, and heart, which did not showed significant differences. The BCS treatment has a significant effect on the Spleen, liver, and kidney. The percentage of these organs increases with CBS level.

The relationship of metabolic rate to body size has become an interesting topic for doctors, especially pediatricians. It has been learned that many quantitative functions vary during growth in relation to metabolic rate, not body size. Example from this was cardiac output, glomerular filtration rate, oxygen
consumption and drug dosage. This phenomenon can reflect direct or possible cause and effect relationships coincidence parallels between a relatively slow increase in metabolic rate compared to body size and function in question. Metabolism decreases and many other measures of physiological function in relation to the unit of body size are widely observed. This phenomenon can be demonstrated by comparing between species, as well as within a species during growth or among members of mature species of varying sizes [7]. Especially in bovine, cow have a basal metabolic rate per kg (BMR/kg) about 69.8 $W^{0.75}$; Cattle, beef heifer 75.4 $W^{0.75}$ and 117.0 $W^{0.75}$ in dairy cattle [8]. In the case of humans during growth, babies have BMR / kg more than double that of normal adults. Normal adults may have a BMR / kg one and a half times that of obese adults. Holliday [7] suggested that high BMR is caused, in part, by a relatively high proportion of body weight taken by all internal organs, including the brain - not because they are large, but their supporting structure.

Spleen, Liver and Kidney were appearing their weight increase depend on the CBS supplementation levels. Spleen is known has important role for antibacterial and antifungal immune reactivity [9]. In the case of responses on anti-nutrient or anti-oxidant affect in metabolic system, various insignificant negative effects on some species were reported widely. Kuku [10] reported that laboratory animals histopathological analysis indicated that the spleen and small intestines were adversely affected, but could have deleterious effects in animals if ingested without adequate processing. Spleen abnormalities, such as large deposition of hemosiderin and melanin pigments and proliferation of melano-macrophage centers, lymphocytic depletion of the white pulp areas (hypocellularity), and presence of vacuoles and necrotic in observed areas were similar to the effects of vitamin E and/or vitamin C deficiencies observed in fish [11]. Some anti-nutritional factors have been observed to inhibit absorption of nutrients and their subsequent utilization and assimilation by animals [12]. Besides, they cause some level of damages to some organs such as liver, kidney and spleen [13].

The condition of hepatotoxicity is a concern in the treatment of CBS supplementation, the high content of active compounds contained therein needs to be a particular concern, because it can affect the performance of organ metabolism nutrition, as theobromine has responsibility for some effect in metabolism [14]. The severity of liver abscesses was evaluated by [15] in individually feeder cows, the report show that concentrate level in the diet of 77.7% affects the severity score of liver abscesses, final live weight (p<.10), hot carcass weight (p<.0001), intake of dry matter (p<.10), increase in daily body weight. In this study, no significant differences between all CBS treatments but significantly higher compared to the control, and the means that the range of 3% of CBS supplementation did not have significant effects on heart size development in bulls. However, [16] workload determines organ size, but dietary factors influencing workload clearly vary for each organ.

The kidneys play to filter the blood, remove wastes, control the body's fluid balance, and keep the right levels of electrolytes. In cattle, which have a higher metabolic rate per unit of metabolic BW than do most other domestic ruminant, but has poorly developed mechanism in the kidney and gut for water retention [17]. In basal metabolism rate (BMR) the proportion of kidney organ was 608±25 g, for cattle with 277,000 ±20,800 BW [18] or about 0.20 – 0.21% BW. The recent study found that CBS on control bulls had no significant differences than those supplemented by 3% of CBS (0.35 vs 0.38), and significantly increase for 0.43 and 0.47% at 6 and 9% of CBS, a greater value than the reference indicates the differences of the cattle breed. Greater value in Bali Bulls may due to their body condition which about two or three times smaller than European breed bulls. Depends on cauture et al. [19], larger mammals have lower tissue metabolic rates (TMR; mumol O2·g wet wt-1·min-1) at 37 degrees C, yielding the equations TMR = 3.6 M-0.21 for liver slices and TMR = 3.2 M-0.11 for kidney cortex slices. Increased the kidney mass can also be influenced by protein intake, Hammod et al [20] concluded that liver, kidney and stomach mass increased with protein intake rate, while digestive tract and other vital organ masses increased only in response to increased energy intake rate. There has not been found any influence or relationship between theobromine or other methylxanthine derivatives directly on kidney performance, but the indirect effect of changes in metabolic activity is very likely to occur.
4. Conclusion
In conclusion, the supplementation of CBS concentrate up to 9% level in the rations did not affect the rumen, gastrointestinal, lung, and cardiac aspiration; but has an effect on the increasing of spleen, liver and, kidneys percentage. Although there was no significant effect on the percentage of the rumen and the digestive tract, there was a tendency in decreasing the percentage of the such two organs weight; indicated the occurrence of degeneration conditions for the long time during the research fattening period.

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