









# EFFECTS OF GRADED LEVELS OF DIETARY CHROMIUM-YEAST ON RUMEN AND BLOOD METABOLITES, FEED DIGESTIBILITY, AND PERFORMANCE OF GOATS

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 Supporting Information

**ABSTRACT:** The study was conducted to determine the effect of dietary supplementation of Chromium-yeast minerals on consumption, feed digestibility, rumen and blood metabolites, Average Daily Gain (ADG) and body condition score (BCS). The research was conducted at Gunung Tugel Farm, Banyumas, Central Java, Indonesia. The material used was 24 male Jawarandu goats with an average initial weight of  $25 \pm 1.23$  kg, individual cages, the feed given consisted of elephant grass silage and concentrate. The treatment feed contains chromium-yeast at levels of 0, 0.5, 1, and 1.5 mg/kg. The research method used was experimental using a completely randomized design. There were 4 treatments tested, namely T0 (70% concentrate + 30% elephant grass silage), T1 (70% concentrate + 30% elephant grass silage + 0.5 mg/kg chromium-yeast), T2 (70% concentrate + 30% elephant grass silage + 1 mg/kg chromium-yeast) and T3 (70% concentrate + 30% elephant grass silage + 1.5 mg/kg chromium-yeast). Each treatment was repeated 6 times so there were 24 trials. The further test used is polynomial orthogonal. The variables measured in this study were feed consumption, feed digestibility, rumen metabolite products, daily body weight gain and body condition score. The results of the analysis of variance showed that the treatment had a significant effect on dry matter and organic matter consumption, feed digestibility, rumen volatile fatty acids (VFA), ammonia nitrogen (NH<sub>3</sub>-N) and blood glucose 3 h post-feeding, chromium-yeast levels, and had a very significant effect on ADG and BCS. In conclusion, chromium-yeast supplementation was able to improve goat performance with optimal levels ranging from 1.04–1.36 mg/kg of feed.

**Keywords:** Body condition score, Chromium-yeast, Daily gain, Goat

## INTRODUCTION

Chromium (Cr) was an essential micronutrient. This mineral was an integral part of Gluco Tolerance Factor (GTF) which increases the adhesion of insulin to the cell membrane surface and allows the entry of glucose, fatty acids and amino acids into cells (Lashkari et al., 2018; Mohanty et al., 2022), due to the binding of these (chromodulin) to the insulin receptor transmembrane (Leiva et al., 2017, 2018; Rode, 2017) specifically to the a subunit, converting it to autokinase via phosphorylation of the b subunit (Vincent and Brown, 2019). Although Cr was essential for insulin function and efficient nutrient metabolism (Assis, 2021) in humans and livestock, consumption of Cr in feed was often less than the recommended dose (0.2 mg Cr/kg DM) (National Research Council, 2005).

Growth hormone (GH) and insulin growth factor (IGF) were involved in Cr metabolism (Lashkari et al., 2018; Moreira et al., 2020) in ruminants, Cr has an impact on growth (Moreno-Camarena et al., 2020; Setyaningrum et al., 2022) Cr increased the response capacity of the immune system (Karaulov et al., 2019); In poultry and swine, Cr supplementation had a better positive effect on metabolic responses, nutrient distribution and carcass characteristics, possibly due to increased sensitivity to insulin and glucose utilization efficiency (Mohanty et al., 2022). The degree of this effect was depend on the dietary intake, as well as the chemical form and concentration of Cr (Genchi et al., 2021; Maret, 2019; Zarczynska and Krzebietke, 2020). The trivalent form of Cr cannot pass the cell membrane, hence its activation requires attachment to a ligand (Sánchez-Mendoza et al., 2015); also some inorganic forms of Cr were not available for uptake (Piray and Foroutanifar, 2022), whereas organic sources were more bioavailable. In sheep and cattle administration rates of 0.2–1.2 mg Cr/kg DM have been tested and doses equal to or greater than 0.35 mg Cr/kg DM has an effect primarily on blood glucose levels. As such, positive effects such as increased growth of lean tissue in ruminants, may be expected from using Cr-enriched yeast. There seems a lack of knowledge on the effects of Cr-yeast on goat fattening, hence further study is required.

The aim of this study was to evaluate the effect of high concentrations of Chromium-enriched yeast added at different levels in a high-energy diet fed to fattening goats on rumen and blood metabolites, feed digestibility, and growth performance.

## MATERIALS AND METHODS

### Ethical approval

Care and treatment of animals in this study were conducted in full compliance with the code of ethics for animal experiments, as outlined in Indonesian Law No. 41 of 2014 concerning Amendments to Law Number 18 of 2009 concerning Animal Husbandry and Animal Health.

### Material

The material used was twenty-four male Jawarandu goats aged 8-10 months ( $25 \pm 0.17$  kg), the goats were dewormed, multivitamins and placed in individual cages ( $1.5 \times 1.2$  m) for an adaptation period of 15 days. Goats were fed basal feed (BF) (Table 1) formulated according to the requirements of finishing goats (National Research Council 2007). The basic feed given was recorded every day and feed samples were taken every week, dried at  $55^\circ\text{C}$  for 48 hours to determine the chemical composition (Table 1). Nutrient level was determined by the Association of Official Analytical Chemists (AOAC) method (2012), dry matter (method 967.03), ash (method 942.05) and crude protein (Kjeldahl procedure, method 976.06, N<sub>6.25</sub>), metabolic energy was calculated based on composition of feed ingredients (National Research Council, 2007). Chromium was given every day and mixed with feed which was served at 08.00. Feed was given ad libitum twice at 08.00 and 16.00. The feed given was recorded every day. Body weight was recorded at the start of fattening and every two weeks during maintenance. On days 14, 35, 49, 63, 77, and 91, three hours after the morning feeding, blood samples (7 mL) were collected from the jugular vein in a vacutainer tube with potassium oxalate. Blood samples were centrifuged and stored at  $-20^\circ\text{C}$  to glucose analysis (Kitchalong et al., 1995) with semi-automated equipment (Vitros DT60II-Johnson and Johnson Co.). Besides, chromium-yeast levels were also analyzed to reveal Average Daily Gain (ADG). Rumen fluid was collected by ribs between 3 and 4 by a veterinarian 4 hours after feeding at the end of the study period.

To ensure consumption of the planned dose, the total daily dose of Cr from each treatment was mixed with 30 g of rejected bread flour and given in the morning using individual feeds. The experiment lasted 105 days. Goats were weighed one by one in the morning (07:00) before feeding. The research design used was a completely randomized design (CRD) with 4 treatments and 6 replications so there were 24 experimental units. The treatment in this study were T0 = basal feed (70% concentrate + 30% elephant grass silage, 15.33% CP and 69.44% TDN); T1 = T0 + 0.5 mg/kg chromium-yeast; T2 = T0 + 1 mg/kg chromium-yeast; T3 = T0 + 1.5 mg of chromium-yeast.

Goats were given feed 4.5% of body weight. Basal feed formulations were presented in Table 1.

**Table 1 - Nutrient Level of Basal Feed**

Feedstuff	g/1000 g DM
Wheat Pollard	308
Rice hull	37.8
Seaweed	25.2
Palm oil meal	105
Soybean Meal	21
Coconut meal	105
CGF	77
Soya shell	35
Mineral mix	10.5
Salt	7
Dolomites	3.5
Kinggrass Silage	300
<b>Nutrients</b>	
DM (%)	67.12
Ash (%)	19.94
Crude Fiber (%)	20.76
Crude Protein (%)	15.06
TDN (%)	66.74
Calcium (Ca, %)	0.6
Phosphorus (P, %)	0.4

CGM: Corn gluten feed, DM: Dry matter, TDN: Total digestible nutrients, Ca: Calcium, P: phosphorus.

### Parameter measurement

Daily body weight gain was obtained by weighing the animals every 2 weeks during the investigation period from the beginning to the end of the study before the animals were given feed. The final result of ADG was obtained by the

difference between the final weight minus the initial weight then divided by the length of maintenance expressed in units of grams/head/day. The formula that was utilized could be explained as follows:

$$ADG = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Days (D)}} = \text{g/head/day}$$

Assessment of the body condition score (BCS) was carried out by the palpation method and observations were made by 3 people as raters. The BCS assessment was carried out by touching the back, base of the tail and hips which were then compared with the BCS table to find out the value.

## RESULTS

The results of this study showed that Chromium yeast supplementation has an effect on the consumption of dry matter and organic matter. The consumption value of dry matter and organic matter was highest in the supplementation of 1 mg/kg dry matter. The results of this study indicated that in the T2 treatment where Jawarandu goats were given basal feed supplemented with 1 mg/kg of chromium-yeast mineral, it was able to produce the highest average value of dry matter and organic matter consumption, namely 1130.23±121.38 g/head/day and 542.28±58.03 g/head/day compared to treatment T0, T1, and T3. The results of this study have lower average consumption of dry matter and organic matter compared to the Moreno-Camarena et al. (2020) who conducted a study adding Chromium yeast up to 0.6 mg Cr-yeast.

Other researchers who conducted research on sheep showed that Chromium-methionine supplementation of up to 3 mg/kg would reduce feed consumption (Seifalinasab et al., 2022), while Kargar et al. (2019) highlight increased feed consumption with chromium supplementation in Holstein male calves. Dry matter consumption was the difference between the dry matter level of the feed given and the dry matter level of the rest of the feed, so that the dry matter consumption of livestock was strongly influenced by the dry matter level of the feed. Consumption of dry matter may also be influenced by the crude fiber level in the ration. The high level of crude fiber in the feed would be difficult to be degraded by rumen microbes so that it has an impact on the digestibility of the feed.

**Table 2 - Effects of chromium-yeast added at different levels in a high-energy diet fed to fattening goats on rumen and blood metabolites, feed digestibility, and growth performance.**

Level of Cr-yeast (ppm)	T0 (Control)	T1 (0.5 mg/kg)	T2 (1.0 mg/kg)	T3 (1.5 mg/kg)	P-value
DMI (g/d)	1014.47±47.30 <sup>ab</sup>	918±99.31 <sup>a</sup>	1130.23±121.38 <sup>b</sup>	1010.28±78.52 <sup>ab</sup>	0.017
DMI/BW (%)	2.99	2.68	3.07	2.88	
OMI (g/d)	486.98±23.61 <sup>ab</sup>	440.55±46.49 <sup>a</sup>	542.28±58.03 <sup>b</sup>	485.35±37.74 <sup>ab</sup>	0.016
OMI/BW (%)	1.44	1.29	1.47	1.38	
CPI (g/d)	141.67±12.43	157.79±20.85	167.90±21.33	157.80±17.68	0.199
CFI (g/d)	183.84±19.36	196.24±23.77	217.93±29.03	188.07±19.71	0.138
DMD (%)	75.37±2.90 <sup>ab</sup>	71.07±1.47 <sup>a</sup>	76.56±2.69 <sup>b</sup>	73.21±2.63 <sup>ab</sup>	0.015
OMD (%)	75.69±3.44 <sup>ab</sup>	71.21±1.59 <sup>a</sup>	76.83±2.84 <sup>b</sup>	73.00±2.38 <sup>ab</sup>	0.022
CPD (%)	82.88±2.34	80.48±3.21	83.98±2.62	82.02±2.51	0.251
CFDs (%)	59.72±6.01	57.20±4.51	66.43±5.18	58.96±4.96	0.360
ADG (g/d)	131.53±10.48 <sup>a</sup>	119.19±16.57 <sup>a</sup>	162.65±3.76 <sup>b</sup>	177.19±20.78 <sup>b</sup>	0.0002
BCS	2.5±0.24 <sup>a</sup>	2.8±0.16 <sup>ab</sup>	2.61±0.18 <sup>a</sup>	3.0±0.22 <sup>bc</sup>	0.0195
VFA (mMol)	73.2±4.72	86.0±9.57	97.4±12.81	99.6±13.15	0.002
NH3-N (mMol)	27.68±1.5	28.72±1.1	27.08±1.67	30.14±1.98	0.09
Glucose (mg/dL)	57.60±8.12	63.43±12.39	60.57±11.36	54.29±8.48	0.067

<sup>a,b</sup>. Means with different superscripts row-wise very significantly. DMI: Dry matter intake, BW : body weight, OMI : organic matter intake, CPI: Crude protein intake, CFI : Crufe fober intake. DMD: Dry matter digestibility, OMD : organic matter digestibility, CPD : Crude protein digestibility, CFD : Crude fiber digestibility. T0 = basal feed (70% concentrate + 30% elephant grass silage, 15.33% CP and 69.44% TDN); T1 = T0 + 0.5 mg/kg chromium-yeast; T2 = T0 + 1 mg/kg chromium-yeast; T3 = T0 + 1.5 mg of chromium-yeast.

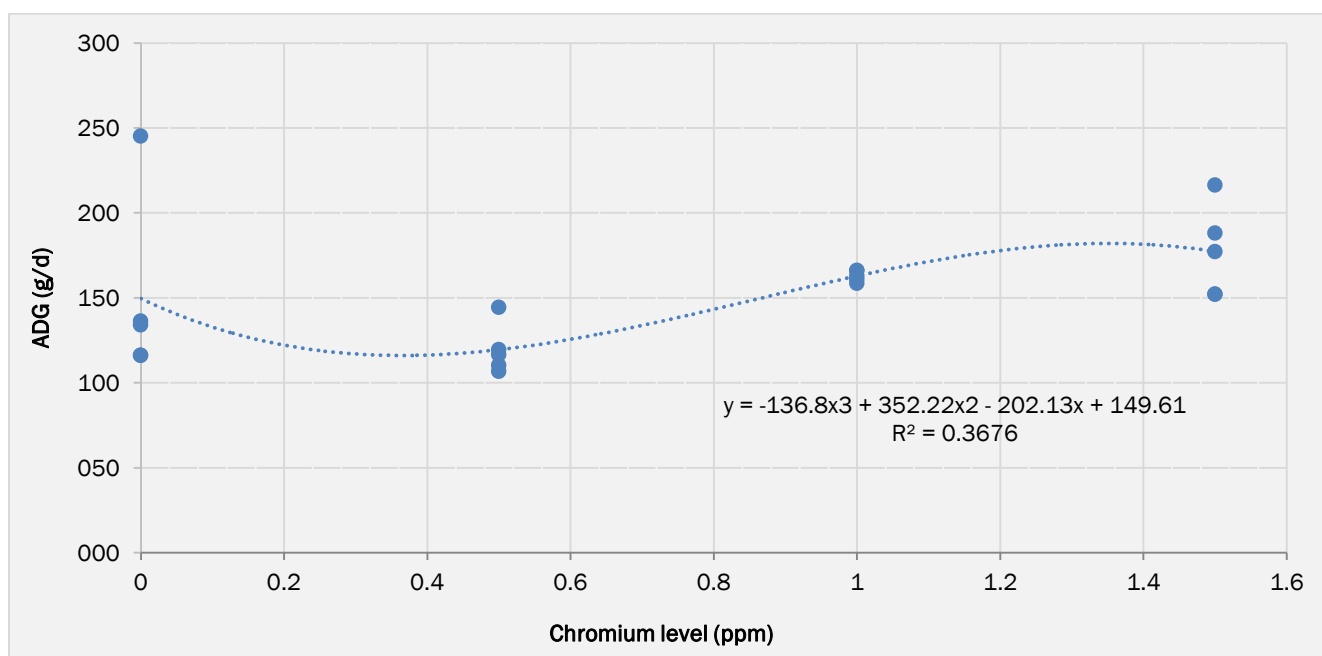
Meanwhile, the consumption of protein and crude fiber in all treatments with the addition of Chromium-yeast showed the same results, which ranged from 141.67±12.43 g/d (T0) to 167.90±21.33 g/d (T2) on protein consumption and 183.84±19.36 g/d to 217.93±29.03 g/d on crude fiber consumption. Even though the effect was not statistically significant, there was a similar trend with the consumption of DMI and OMI in that the T2 treatment (addition of 1 mg/kg) showed the highest consumption of protein and crude fiber. Feed containing chromium could increase the fermentation

of the ration in the rumen because chromium was an essential mineral for the growth, performance and population of rumen microbes so that the fermentability in the rumen increases (Prayitno et al., 2014; Setyaningrum et al., 2022).

Based on the average value of the digestibility of dry matter and organic matter in Table 3, it showed that the treatment has a significant effect on the digestibility of dry matter and organic matter. The results of this study indicated that in the 1 mg/kg Chromium-yeast supplementation treatment where Jawarandu goats were given basal feed supplemented with organic chromium mineral 1.0 mg/kg of feed was able to produce the highest average digestibility of dry matter and organic matter, namely  $76.56 \pm 2.69\%$  and  $76.83 \pm 2.84\%$  compared to the 0, 0.5 and 1.5 mg/kg Chromium-yeast treatments. Digestibility of dry matter was the difference between the amount of dry matter consumption minus the dry matter of faeces divided by the amount of dry matter consumption multiplied by one hundred percent, while the digestibility of organic matter was the difference between the amount of consumption of organic matter minus the organic matter of faeces divided by the amount of organic matter consumption multiplied by one hundred percent.

The results indicated that chromium-yeast supplementation has an effect on daily gain, body condition score, rumen volatile fatty acids, ammonia nitrogen and blood glucose. Chromium as a glucose tolerance factor was able to stimulate the hormone insulin to carry glucose to target organs. It was characterized by the rise and fall of glucose concentrations. Chromium-yeast supplementation increased the concentration of total rumen Volatile Fatty Acids. Rumen volatile fatty acids was the main source of energy in ruminants, thus increase in VFA has a positive impact on growth. Chromium-yeast supplementation 1–1.5 mg/kg gives the most response on daily gain. It was believed that the availability of energy in the form of VFA was also balanced with the optimal availability of N sources (in the form of  $\text{NH}_3\text{-N}$ ). The fatty acid component of VFA which greatly influences growth was propionic acid. Propionic acid has glucogenic properties which in the liver would be converted into blood glucose. Blood glucose would enter the cells and be used to support the body's fat and protein synthesis as well as a source of ATP (He, 2022). The function of organic chromium added to the feed may help speed up the process of glucose transportation in the livestock body. Organic chromium could increase the activity potential of the insulin hormone which plays an important role in the transport of glucose and amino acids. Increased use of glucose by insulin would then be used by livestock for organ formation, namely the formation of muscle and adipose tissue.

Chromium level result was different with as already mentioned. As such, it can be seen that chromium level was affected by the treatment performed. These results showed that Chromium had normal and higher total VFA levels compared to goats not given organic Chromium.



**Figure 1 - Average daily gain (ADG) result.**

## DISCUSSION

Based on the polynomial orthogonal further test results that the use of chromium levels to produce the maximum average daily gain (ADG) was achieved at the level of 1.36 mg/kg. Using chromium at this level results in ADG which was at the top of the curve (Figure 1). The high ADG of goats was supported by the age of the goats used. Goats used as research material have ages ranging from 8-10 months, at this age there was rapid growth, before sexual maturity and slow growth occurs when the body was mature.

The high ADG in the T3 treatment, which was  $177.19 \pm 20.78$  g/head/day, was due to the provision of good quality feed. The feed given was in the form of 30% forage and 70% concentrate. The feed produced a CP of 15.33% and a TDN of 69.44%. Based on the SNI table for fattening goat feed, the feed given was according to the standard. The high ADG results was supported by chromium-yeast supplementation at a level of 1.5 mg/kg feed. Giving chromium-yeast in the feed would accelerate the digestibility of the feed so that the absorption of feed nutrients for the formation of meat becomes more optimal. The use of Cr-yeast was thought to increase the proportion of propionate. the addition of organic chromium increased the production of ammonia (NH<sub>3</sub>), total VFA and the proportion of propionate in the rumen (Abdel-Raheem and Hassan, 2021). Propionic acid has glucogenic properties which in the liver would be converted into blood glucose. In this case, blood glucose would enter the cells and be utilized to support the body's fat and protein synthesis as well as a source of ATP (He, 2022; Sultana et al., 2022). The function of chromium added to the feed may help speed up the process of glucose transportation in the livestock body. Chromium-yeast could increase the activity potential of the insulin hormone which contributed in the transport of glucose and amino acids. Increased use of glucose by insulin would be used by livestock for tissue formation, namely the formation of muscle and adipose tissue (Setyaningrum et al., 2022; van der Kolk et al., 2017).

The results of this study also indicated that the treatment given has a very significant effect on the goat's BCS. The highest BCS was achieved in the T3 treatment which was  $3.00 \pm 0.19$ . This is was when compared to the study of Ahreza et al. (2020), which has a BCS of  $2.64 \pm 0.37$ . These results highlight that the study goats showed ideal body conditions. The ideal BCS indicated that the feed given was of good quality and the management applied was appropriate. The lowest BCS value was achieved in the T0 treatment with an average of  $2.5 \pm 0.24$ . This indicated that the T0 feed in the form of elephant grass silage + concentrate has not been able to increase BCS to the fullest. However, this figure was still within the normal/ideal BCS range. BCS value in the T1 treatment ( $2.8 \pm 0.26$ ) higher than the T2 treatment ( $2.6 \pm 0.15$ ). However, this was inversely proportional to the ADG. Treatment on T2 resulted in greater ADG than T1 ( $162.89 \pm 3.87$  vs  $119.50 \pm 17.12$ ). The difference in BCS was caused by many factors, one of which was age. According to Ghosh et al. (2019), as goats get older, goats may lose body fat quickly. This was in line with the fact that the goats used as research material had ages in the range of 8-10 months. The BCS value in the T2 treatment (1 mg/kg) was lower than the T1 treatment (0.5 mg/kg) which may also be suspected because of the lower fat level in T2 goats.

The mean values of BCS T0, T1 and T2 were in the range of 2.5. This figure was still within the normal range and gets the ideal predicate. Visualization that appears in goats was a slightly bony appearance, the backbone was quite visible, the spinous bones look slightly protruding and one third of the transverse bones were visible. When palpating, the transverse bones could be easily felt by the fingers with pressure. The ribs could be felt, the intercostal spaces were smooth but permeable, the muscle areas were of moderate depth and little overlying fat. Factors that affect the BCS of goats include genetics, feed, and applied management.

The mean value of the BCS T3 treatment would be the pressure required to feel the spine. The transverse and spinous bones may be felt by the fingers and there was moderate muscle and fat tissue when pressure was applied. The visual appearance of the goat was somewhat rounded, the spine was not very prominent, the ribs were not clearly visible and the intercostal space could still be felt with a little pressure. Based on the results of the BCS assessment, goats in the T3 treatment received an average rating.

Based on Table 4, treatment T0 where goats were fed basal diet without Chromium resulted in total volatile fatty acids (VFA) levels with an average lower than normal levels, namely below 80 mM, whereas in treatments T1, T2, and T3 where goats were given feed supplemented with organic Chromium produced higher levels Total volatile fatty acids (VFA) with an average that was within normal levels, namely between 80-160 mM. These results showed that in this study goats fed organic chromium had normal and higher total VFA levels compared to goats not given organic Chromium. This was in accordance with the research of Jayanegara et al. (2006) that Chromium supplementation in both inorganic and organic forms resulted in total VFA production within the optimal and proper range for the survival of ruminants, namely between 80–160 mM.

Normal levels of total VFA in goats fed chromium-yeast at T1, T2, and T3 with successive doses of 0.5, 1 and 1.5 mg/kg indicated that the administration of Chromium- Yeast at this dose was safe for the feed fermentation process by rumen microbes. Normal volatile fatty acids (VFA) levels indicated that the feed fermentation process that occurs in the rumen was going well (Sutaryo et al., 2019). This was supported by statements by Prayitno et al. (2014) that the better the growth of rumen microbes may cause the population of carbohydrates digesting microbes to be higher, this causes the process of carbohydrate fermentation in the rumen to run better.

Ammonia nitrogen (NH<sub>3</sub>-N) in the rumen fluid was the result of the degradation process of protein and non-protein nitrogen (NPN) in the rumen which plays a role in the synthesis of rumen microbial protein. Ammonia nitrogen was used by rumen microbes as the main source of nitrogen to synthesize protein. The concentration of NH<sub>3</sub>-N in the rumen was an indicator to determine feed fermentability which was related to feed protein digestibility, activity and rumen microbial population. Based on the results of all treatments in this study, the lowest average concentration of NH<sub>3</sub>-N was 29.2 mM and the highest total NH<sub>3</sub>-N level was equal to 30.4 mM. This figure was higher than Jayanegara et al. (2006) with NH<sub>3</sub>-N concentrations of 9.97-13.28 mM in in vitro feeding supplemented with Chromium in organic and inorganic forms. According to Evyernie et al. (2019), the optimal NH<sub>3</sub>-N concentration that supports rumen microbial protein synthesis

was 6-21 mM. The high concentration of NH<sub>3</sub>-N indicated that some of the feed protein sources were easily degraded, however, concentrations up to 30.15 mM were still safe for the growth of rumen microbes and hosts.

## CONCLUSION

From the results of the study, it was concluded that Chromium supplementation in goat feed affects feed consumption, feed digestibility, rumen and blood metabolites, average daily gain and body condition score. The best Chromium-yeast supplementation in optimal fattening goat feed at the level of 1.04-1.36 mg/kg feed.

## DECLARATIONS

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### Authors' contribution

W.Suryapratama, Munasik, T.Widiyastuti, E.Susanti performed conceptualization, data curation, formal analysis, investigation, methodology, software, validation, writing original draft; P.Yuwono performed conceptualization, methodology, supervision, writing original draft, review and editing of the manuscript for important academic contents.

### Conflict of Interest

The authors have not declared any conflict of interest

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