

Original Research Paper

# Effect of a Phytomineral Complex and an Enzyme Preparation on the Absorption of Nutrients and the Energy Balance of Calves' Diet

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**Abstract:** Cost-effective animal husbandry directly depends on the correct breeding of replacement young animals using diets corresponding to the feeding rate for all nutrition elements of the detailed system. Critical to these diets are biogenic elements, pivotal in enzymatic reactions and immune defense. However, specifics on enhancing nutrient absorption through dietary supplements remain underexplored, particularly in the context of calves' preweaning growth. This study aims to evaluate the impact of a phytomineral complex and an enzyme preparation, GlucoluxF, on the digestibility and assimilation of nutrients in preweaning calves' diets. This scientific and economic experiment was performed on 64-month-old black-and-white breed heifers divided into four groups with balanced randomization to ensure representativeness. Each group was kept based on the group method and received the same diet following the keeping technology adopted on the farm. Apart from the main diet received by all the experimental groups, the second group additionally received a phytomineral complex at the rate of 100 mL per head per day, the third group received an enzyme preparation GlucoluxF at the rate of 500 g per t of compound feed and the fourth group received both feed supplements in the same doses. The obtained data allowed us to establish the effectiveness of the combined use of the feed supplements in the diet of the replacement young animals, which activated the processes of digestion and assimilation of nutrients in the diet and increased the amount of pure energy for the anabolic processes of the body. Findings suggest that the strategic addition of a phytomineral complex and GlucoluxF to the diets of preweaning calves can significantly enhance the digestibility and assimilation of nutrients. This study contributes to the field by providing a detailed examination of the effects of these dietary supplements, underscoring their potential value in advancing nutritional strategies for young livestock.

**Keywords:** Digestibility, Nutrients, Energy, Calves' Diet, Phytomineral Complex, Enzyme

## Introduction

Today, in cattle breeding, the main condition for increasing cattle productivity is a full and balanced feeding containing all organic substances and nutrients (Gorlov *et al.*, 2020). In this field, Atlanderova *et al.* (2024) identified endemic zones with deficiencies of manganese, copper, cobalt, zinc and iodine and proposed the norms for the introduction of trace elements, the use of which can significantly increase the productivity of farm animals, thereby the profitability of their breeding.

The lack of trace elements in the cattle diet causes disturbances in the vital activity of the body and leads to a decrease in the productivity of farm animals (Makaeva *et al.*, 2019). One can increase the degree of use of the bio elements in various ways, one of which is combination with other biologically active supplements of amyl-, lipolytic and proteolytic action, as well as phytobiotics, the use of which has increased in recent years (Pozdnyakova and Kostomakhin, 2021).

It is possible to make up for the lack of elements by introducing salts of nitrites, nitrates, or oxides into the diet, which is the easiest way to adjust it to the animals' needs. However, it is impossible not to consider the degree of their dissociation and bioavailability in the body (Kulmakova *et al.*, 2021). The most digestible form is possessed by biogenic elements associated with a molecule of organic matter (hexoses) and amino acids (Sleptsov *et al.*, 2021). This complex of chelated compounds will serve as activators of all metabolic processes of the body with a complex positive effect on the activity of the endocrine glands and the beneficial microflora of the gastrointestinal tract (Duskaev *et al.*, 2024a).

The use of phytopreparations made of various medicinal herbs, tree bark is increasingly used in animal husbandry as an alternative to antibiotics (Gorlov *et al.*, 2020). Considering the fact that many trace elements are part of enzymes and vitamins and increase their functional activity in the body, the inclusion of proteolytic enzymes in the diet can increase the retention of feed nutrients in products, act as catalysts for metabolic processes, immunomodulators and immunostimulators and significantly reduce feed costs per unit of live weight gain (Shirina *et al.*, 2024; Soslanovna, 2021; Sultanaeva and Baldzhi, 2024).

Exogenous enzymes act as a means of improving the consistency, nutritional value and efficiency of feed used by ruminants by increasing the digestibility of fiber and reducing the action of antinutrients (Abid *et al.*, 2021).

Conducting experiments on the study of metabolism is aimed at determining the degree of digestibility of nutrients and energy use in ruminants (Pozdnyakova and Kostomakhin, 2021).

Despite the recognition of the critical role played by biogenic elements in supporting enzymatic reactions and immune defense mechanisms, there exists a notable research gap in exploring innovative ways to enhance the bioavailability and efficacy of these elements in livestock diets. Specifically, the combination of trace elements with other biologically active supplements, such as phytominerals and enzyme preparations, represents an underexplored area with the potential to revolutionize dietary strategies for young animals, especially calves during their preweaning growth period.

Addressing this gap, our study focuses on investigating the synergistic effects of a phytomineral complex and an enzyme preparation, GlucoluxF.

Therefore, the main purpose of our study was to find out the effect of the enzyme preparation GlucoluxF and the phytomineral complex on the digestibility and assimilation of nutrients in the diets of calves during the preweaning growing period.

Firstly, it addresses the pressing need for innovative solutions to nutrient deficiencies and challenges in animal feeding practices. Secondly, it offers a clear articulation of how combined dietary supplements can significantly impact the nutritional management strategies employed in livestock farming.

## Materials and Methods

The protocol of the study was discussed and approved at a meeting of the local bioethics commission of the Kostanay Regional University named after A. Baitursynov of the science committee of the ministry of education and science of the Republic of Kazakhstan (protocol no. 3 dated June 21, 2021). The approval adheres to the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan's guidelines for animal research. Specific attention was paid to ensuring that all aspects of the study respected animal welfare considerations.

The scientific and economic experiment in studying the effect of mineral phytocomplex and enzyme preparation on the level of assimilation of nutrients in the diet was conducted at the agricultural enterprise Nizhnyaya Sanarka LLC (Chelyabinsk region, Russia) from November 5 to November 20, 2020. The experiment was carried out on four groups (n = 60) of black-and-white breed heifers of the preweaning growing period, based on their weight and physiological condition to ensure homogeneity across the experimental subjects. The calves were then randomly divided into four groups of 15 animals each to eliminate selection bias and ensure each group was representative of the whole population. This randomization process involved assigning each calf a unique identifier and using a computer-generated random number sequence to allocate the calves to one of the four study groups.

The actual intake of nutrients by calves of the control and experimental groups was: Skimmed milk: 5.5 kg, brome hay: 0.99-1.05 kg, haylage: 1.35-1.65 kg, compound feed: 0.97-1.1 kg, table salt: 15 g and diammonium phosphate: 16 g/head per day.

Calves of the second group, in addition to the main diet, received 100 mL of the phytomineral complex, which was given with the milk feed. The phytomineral extract was obtained by hydrobarothermal treatment of alfalfa hay and enriched with copper, manganese, cobalt and iodine from a calculation of 10-50 mg per 100 kg of live weight.

The method of stepwise mixing per ton of combined feed for the calves of the third group was used to obtain 500 g of the enzyme GlucoluxF, which has amylolytic and cellulolytic activity due to the content of glucoamylase (at least 1,000 units/g) and xylanase (up to 600 units/g). The calves of the fourth group received combined the same amount of tested feed supplements as in the previous experimental groups.

Due to the formulation of the digestion experiment, the degree of digestibility of organic substances and the energy balance of the animal diet were determined. The accounting and preparatory periods equaled 15 days. Three calves were selected from each group. They were placed in individual stalls. A fecal bag and a urine receiver with a hose were attached to each animal, diverting the excreted urine into a bottle. The selected stool samples were preserved with a 10% hydrochloric acid solution at a dosage of 100 mL per 1 kg of feces. The urine samples were preserved with a 10% hydrochloric acid solution so that its total amount was 5% of the sample weight. Then 2 g of thymol was added. Besides, urine samples were periodically checked for litmus to monitor the pH level of the samples and a hydrochloric acid solution was added to a slightly acidic reaction. The obtained samples were stored in the refrigerator at a temperature of +3°C.

Furthermore, the average feed samples were taken for chemical analysis. Sampling was carried out separately from each daily collected material. Concentrated feed (200 g), 400 g of coarse feed and 2 kg of succulent feed were sent for research. The samples were placed in jars with lids. Feed residues were also recorded for each animal separately (Ovsyannikov, 1976).

To determine the chemical composition, the selected samples were sent to the interdepartmental educational laboratory of the South Ural State Agrarian University in Troitsk (Chelyabinsk region, Russia), where they were examined using atomic adsorption method on a quantum-2M spectrophotometer.

To determine the energy balance in the calves' body, the amount of energy received with feed and its excretion

from the body with urine, gases and feces were determined. The difference between the received and released energy shows the energy value of proteins, fats and carbohydrates in the metabolism of organisms. The balance of energy in the body of animals depends on its concentration in the diet. The calculation of Gross Energy (GE) in the diets was carried out based on the chemical composition of feed and energy coefficients for each type of nutrient: For protein 23.95, for fat 39.77, for fiber 20.05 and for Nitrogen-Free Extractive Substances (NFES) 17.46 MJ per 1 kg of substance.

Thus, GE was determined using the following formula:

$$GE = 23.95 \times \text{crude protein (cP)} + 39.77 \times \text{crude fat (cFa)} + 20.05 \times \text{crude fiber (cFi)} + 17.46 \times \text{cNFES, (MJ)}$$

The energy of Digestible Nutrients (DN) was calculated using the formula:

$$DN = 24.24 \times \text{digestible protein (dP)} + 34.12 \times \text{digestible fat (dFa)} + 18.51 \times \text{digestible fiber (dFi)} + 17 \times \text{pNFES, (MJ)}$$

Physiologically useful energy of the calves' diet (metabolizable energy, Me) was obtained by eliminating energy loss with feces, urine and intestinal gases and was determined using the formula proposed by the all-union academy of agricultural sciences (VASHNIL):

$$Me = 17.46 \times dP + 31.23 \times dFa + 13.65 \times dFi + 14.78 \times \text{pNFES, (MJ)}$$

The following formulas were used to calculate the amount of Pure energy to maintain life (*Peml*) and metabolizable energy to Maintain life (*Meml*):

$$Peml = 5.67 - 0.061 \times M$$

$$Meml = Peml : 0.55 + 0.016 \times MEC$$

where, *M* is the mass of the animal, kg *MEC* is the metabolizable energy concentration in 1 kg of Dry Matter (DM) of the diet, *MJ*: kg of DM.

The amount of metabolizable energy above Maintaining life (*Meaml*) was determined using the formula:

$$Meaml = ME - Meml, (MJ)$$

The value of the Net Gain Energy (NGE) was determined using the formula:

$$NGE = 0.035 \times MEC \times Meaml$$

The results of the experiment were subject to biometric and mathematical processing using Microsoft Office and statistica 10.0 the determine the level of reliability of the data obtained.

## Results

During the experiment, we determined that the studied feed supplements changed the intake of nutrients from the diet of animals (Table 1).

Table 1 shows that the consumption of dry matter in the third group was 18.3% higher ( $p < 0.05$ ) and in the fourth group 20.5% higher ( $p < 0.05$ ) than in the control group. The organic matter indicator in the third group was higher by 18.1% and in the fourth group by 20.1%. A smaller difference in these indicators was observed in animals of the second group (14.2 and 14.1%) in comparison with the control group. A similar pattern can be traced for the components of the organic part of the feed. However, there is no statistically significant difference between the control and experimental groups.

The content of undigested nutrients in fecal masses selected from three calves of each group is presented in Table 2.

Analyzing Table 2, one can notice a tendency to increase the loss of DM in the feces of calves of the experimental groups in comparison with the control group. This can be explained by an increase in the ash content index, while the loss of organic matter did not

have a significant difference in the results obtained, since it was within the error of the arithmetic mean. However, in animals of the third and fourth groups, there is a significant decrease in cP losses and a similar trend is seen in cFi in the second and third groups. However, the indicators of reducing the cFa loss in all groups were close in value. In the second and third groups, with the addition of the phytomineral complex and the enzyme to the diet of animals, there was a tendency for an increase in the excretion of NFES from the body.

The data on the amount of digested nutrients are presented in Table 3.

The obtained data from Table 3 indicate that the calves of the experimental groups tended to increase the intake of DM and the organic part of the diet in comparison with the control group. Moreover, the intake of cP in the first experimental and control groups had the same indicator (374.74 and 374.38 g), while in the third and fourth groups, it was higher by 5.8 and 6.3%. The intake of cFi in the experimental groups was higher than in the control group by 6.8-29.1% and for CFA, this difference equaled 9.9-13.7%. The increase in NFES was noted only in the third and fourth experimental groups.

**Table 1:** Intake of nutrients into the body of calves with the diet, g ( $X \pm m_x$ , n = 3)

Indicator	Group			
	I	II	III	IV
DM	2,375.60±215.62	2,713.65±3.33	2,809.69±17.42*	2,862.93±67.25*
Organic matter	2,237.15±202.15	2,551.61±3.72	2,641.37±16.06	2,687.83±62.32
cP	516.02±006.57	510.81±2.90	527.64±03.91	524.39±03.33
cFi	502.90±006.08	504.15±5.04	528.94±08.36	566.11±24.39
cFa	84.80±005.00	87.64±0.51	91.11±00.74	91.12±01.36
NFES	1,358.02±118.23	1,449.01±5.06	1,483.72±16.41	1,506.21±33.53

**Table 2:** The content of undigested nutrients in calves' feces, g ( $X \pm m_x$ , n = 3)

Indicator	Group			
	I	II	III	IV
DM	686.76±32.05	729.70±04.99	727.30±9.60	712.39±11.85
Organic matter	600.96±20.53	619.92±09.59	605.74±8.43	593.56±12.02
cP	141.28±01.33	136.43±01.37	131.00±1.11***	126.09±03.66***
cFi	280.02±07.12	266.05±03.24	265.30±2.21	278.49±03.67
cFa	40.53±02.12	38.99±00.73	41.19±0.59	040.79±01.04
NFES	139.14±18.57	178.46±10.83	168.25±9.68	148.19±12.37

**Table 3:** Digested nutrients of calves' diet, g ( $X \pm m_x$ , n = 3)

Indicator	Group			
	I	II	III	IV
DM	1,688.84±185.65	1,983.95±006.54	2,082.39±26.81	2,150.54±56.47
Organic matter	1,636.19±188.12	1,931.68±013.24	2,035.64±22.96	2,094.27±52.37
cP	374.74±007.68	374.38±003.96	396.64±2.830	398.30±06.19
cFi	222.88±006.52	238.10±007.02	263.64±7.96***	287.62±20.74**
cFa	44.27±002.89	48.65±001.24	49.92±1.080	50.33±02.22
NFES	1,218.88±100.86	1,174.30±111.46	1,315.46±25.31	1,358.02±25.38

**Table 4:** Coefficients of digestibility for the nutrients in the calves' diet, % ( $X \pm m_x$ , n = 3)

Indicator	Group			
	I	II	III	IV
DM	70.83±1.34	73.11±0.19	74.11±0.50	75.11±0.25*
Organic matter	72.83±1.77	75.70±0.41	77.06±0.44	77.91±0.26*
cP	72.60±0.55	73.29±0.39	74.84±0.35*	75.95±0.79*
cFi	44.33±1.20	47.21±0.97	49.82±0.76**	50.68±1.44**
cFa	52.17±0.34	55.50±1.09*	54.78±0.84*	55.19±1.65
NFES	89.83±0.65	86.52±1.93	88.64±0.78	91.64±1.28

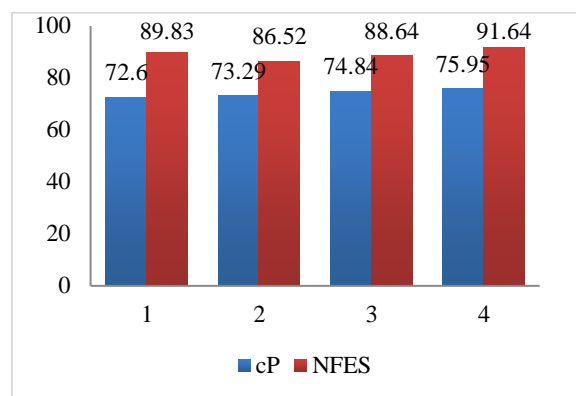
**Table 5:** Energy balance in the body of calves, on average MJ/head per day ( $X \pm m_x$ , n = 3)

Indicator	Group			
	I	II	III	IV
GE of the diet	49.52±02.50	49.45±01.75	52.77±00.50	53.83±01.20
Energy of digestible nutrients	35.44±02.03	36.46±00.53	38.52±00.57	40.15±00.90
ME of the diet	29.04±01.76	29.78±00.44	31.48±00.49	32.85±00.72
Peml	12.44±00.22	12.50±00.14	12.62±00.11	12.79±00.12
Meml	22.82±00.39	22.90±00.25	23.13±00.19	23.43±00.27
Meaml	06.22±02.12	06.93±01.87	08.35±00.52	09.41±00.48
NGE	02.70±00.94	02.68±00.31	03.28±00.23	03.78±00.19
Digestibility coefficient, %	71.06	73.07	73.00	74.60
Metabolism coefficient, %	58.06	60.02	59.07	61.00
Coefficient of productive use, %	05.04	05.04	06.02	07.00

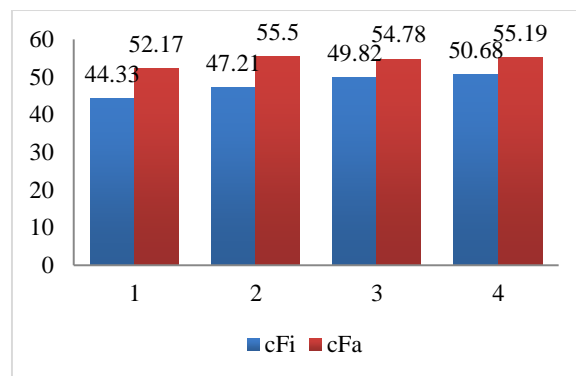
From the obtained results of the intake of dietary nutrients into the body of experimental animals and the degree of their digestibility, it is possible to determine the coefficient of digestibility (Table 4 and Figs. 1-2). According to Table 4 and Figs. 1-2, in the group where the phytomineral supplement was used together with the enzyme, there was an increase in the digestibility of cFi by 2.88%, cFa by 3.33% and cP by 0.69% compared to the first control group.

The calculation we obtained on the degree of utilization of the GE of the calves' diet at the level of ME and productive energy is presented in Table 5.

Table 5 shows that the GE of the calves' diet in two experimental groups with the amylolytic enzyme, as well as together with the phytomineral complex, was 52.77±0.50 and 53.83±1.20 MJ/head per day, which is 6.6 and 8.7% higher than the control group, respectively. The DN energy in the second group was 36.46±0.53 MJ/head per day, which is higher than the control by 2.9%; in the third group 38.52±0.57 MJ/head per day, higher than the control by 8.7% and in the fourth 40.15±0.90 MJ/head per day, higher than the control by 13.3%. This trend can be explained by the higher digestibility of proteins, fats, fiber and NFES under the influence of the phytobiotic and the amylolytic enzyme. The ME indicator in the second group was higher than the control group by 2.5%, in the third by 8.4% and in the fourth by 13%.



**Fig. 1:** Coefficients of digestibility of cP and NFES in the calves' diet, %



**Fig. 2:** Coefficients of digestibility of cFi and cFa in the calves' diet, %

There are also differences in the further distribution of the Meml of the diet, heat gain and the formation of products. The Peme and Meml indicators in the second group differed slightly from the control group; in the third group, they were higher by 1.4-1.5% and in the fourth group by 2.8-3%. However, the Mame in calves of the second group was 11.4% higher than the control group; in the third group, it was higher by 34.2% and in the fourth group by 51%. As a result, the NGE in calves in the second group did not differ from the indicator of the control group and amounted to 2.68-2.7 MJ; in the third group, it equaled 3.28 MJ or 21.5% higher than the control group and in the fourth group, 3.78 MJ or 40% higher.

The calculations of the coefficients of digestibility, metabolism and productive use of energy in the calves' diet also had differences. Thus, the coefficient of digestibility of the GE of the calves of the second group was 2.1% higher than the control group and amounted to 73.7%; in the third group, it was 1.4% higher and in the fourth group 3% higher. The most significant difference in the metabolism coefficient was obtained in the fourth group (61.0%), which was 2.4% higher than the control group. Consequently, the coefficient of productive energy use of the animal diet in the second group was equal to the control group (5.4%), while in the third group, it was 6.2% higher and in the fourth group 7% higher.

Based on the calculations obtained on the energy use of the calves' diet and the high coefficient of productive action, in the experimental group with the combined use of the phytomineral complex and the probiotic enzyme GlucoluxF, better digestion and assimilation of all the necessary nutrients in the diet were observed.

## Discussion

The nutrients needed by the body for metabolic processes associated with the synthesis of new tissues and the development of internal organs of all systems are determined by the difference in the amount of nutrients received and excreted from the body (Sannikova *et al.*, 2021).

The functional properties of the enzyme supplement under study are aimed at enhanced use of both simple and complex carbohydrates by the body. In all likelihood, the enzyme activated the work of the endocrine glands, in particular, the pancreas and along with this, proteolytic and lipolytic activity (Soslanovna, 2021). As a result, in the third group, the difference in the digestibility of cFi with the control group was 5.49%. The digestibility of cP in the same group was higher than the control by 2.24% ( $p < 0.05$ ), the digestibility of cFa by 2.51% ( $p < 0.05$ ) and the digestibility of NFES slightly differed from the control group.

In the experimental group of calves, where a phytomineral complex containing copper, zinc, cobalt, manganese and iodine was additionally received with the main diet, a difference in the digestibility of DM and the organic part of the feed was observed, which indicates activation of the enzymatic activity of the body's endocrine glands (Favaretto *et al.*, 2020).

The synergistic effect of the exogenous enzyme and a complex of trace elements contributed to strengthening the processes of digestion of nutrients in the fourth experimental group. In this group, the digestibility of CP was higher than the analogs of the control group by 3.35% ( $p \leq 0.05$ ), the digestibility of cFi was higher by 6.35% ( $p \leq 0.01$ ), the digestibility of cFa by 3.02% and the digestibility of NFES by 1.81%. Similar results were obtained by Nurzhanov *et al.* (2022) with the introduction of an additional amount of highly dispersed manganese particles, as well as the case of the addition of highly dispersed copper, zinc and calcium particles to the diet of young cattle reported by Marina Yakovlevna *et al.* (2018).

The results show the difference in the digestibility of the organic part of the calves' diet, which in the animals of the second experimental group exceeded the control group by 2.87%, in the third group by 4.23%, in the fourth group by 5.08% ( $p < 0.05$ ) and in general in DM by 2.28, 3.28 and 4.28% ( $p \leq 0.05$ ), respectively. This aligns with the trajectory of recent research emphasizing the critical role of dietary formulations in enhancing livestock nutrient absorption. Specifically, Robles Jimenez *et al.* (2023), found that the strategic inclusion of fibrolytic enzymes in ruminant diets significantly boosts the breakdown and assimilation of complex feed components. Our findings contribute to this growing body of evidence, suggesting that both the type and combination of dietary supplements can have a compounded effect on the efficiency of nutrient use, thereby enhancing the overall health and growth potential of young livestock.

Also, the positive effect on the digestibility and assimilation of nutrients in the diet from the use of glucoamylase together with oak bark extract was proved by Duskaev *et al.* (2024b).

## Conclusion

As a result of our study, we determined that the greatest positive effect in the digestibility of nutrients and the assimilation of the organic part of the diet, as well as a higher coefficient of productive use of the energy of the diet, is observed when the phytomineral complex and the amylolytic enzyme GlucoluxF are used together in the calves' diet. Specifically, the calves receiving both supplements exhibited a notable improvement in nutrient

digestibility and energy assimilation, with increases in the digestibility coefficients for crude protein, crude fiber and non-fibrous extractive substances by up to 3.35% ( $p < 0.05$ ), 6.35% ( $p < 0.01$ ) and 1.81% respectively, compared to the control group. Additionally, the combined supplement group showed an increase in energy balance metrics, indicating a more efficient utilization of the diet provided.

Such results suggest that the integration of these supplements into calves' diets can potentially increase feeding efficiency, nutrient absorption and ultimately, the growth and productivity of animals.

It is important to acknowledge potential limitations within our study. This study was conducted over a relatively short time with a limited sample size and more research is needed to confirm these results over longer periods and on larger populations.

Future research in this area can focus on the economic and environmental implications of including these supplements in animal husbandry practices, providing a fuller understanding of their feasibility and sustainability.

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## Author's Contributions

All authors equally contributed to this study.

## Ethics

This article is original and containing unpublished material. The corresponding author affirms that all co-authors have reviewed and approved the manuscript and there are no ethical concerns.

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