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Determination of Heavy Metals in the Liver of Dairy Cows and the Risk to their Health

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Abstract: Heavy metals are characterized by their high atomic mass and toxicity to living organisms. This study aimed to investigate the presence of heavy metals in the livers of slaughtered dairy cows and to discuss their possible effects on animal health. In the study, 50 Holstein dairy cows were used. After slaughtering, samples of 4 X 10 g were taken from the liver of the animals for heavy metal analysis. Samples prepared according to the wet burning method were analyzed for the presence of arsenic (As), aluminum (Al), lead (Pb), mercury (Hg), and nickel (Ni) using the ICP-OES device. In clinical examination, rumen acidosis and ovarian diseases were detected as the most common diseases in 20 and 13 animals, respectively. None of the samples contained Ni. On average, Al was detected at 4.60±8.71 ppm, As at 0.39±0.11 ppm, Hg at 0.41±0.29 ppm and Pb at 0.04±0.13 ppm. Based on the total number of animals, the following prevalence was calculated: 72% for Al, 100% for As, 88% for Hg, 0% for Ni, and 10% for Pb. The study showed that the average As content was in a toxic range. In 28% of the samples, the Al value was categorized as toxic to animal health. The average Hg values, on the other hand, were above the acceptable limits for human health. In summary, the prevalence of toxic heavy metals Al, As and Hg in Holstein livers was quite high.
Keywords: *Aluminium, Arsenic, Heavy metal, Lead, Liver, Mercury.*

Süt İneklerinin Karaciğerlerinde Ağır Metal Varlığı ve Sağlık Riski

Özet: Ağır metaller, yüksek atom ağırlıkları ve canlılar üzerine olan toksisiteleri ile bilinirler. Bu çalışma, kesilen süt sığırlarının karaciğerlerinde ağır metallerin varlığını ve bunların hayvan sağlığına olası zararlarını araştırmayı amaçlamıştır. Bu çalışmada 50 Holstein süt ineği kullanılmıştır. Ağır metal analizi için, kesimden sonra hayvanların karaciğerinden 4x10 g örnekler alındı. Yaş yakma yöntemine göre hazırlanan örnekler, ICP-OES cihazı kullanılarak arsenik (As), alüminyum (Al), kurşun (Pb), civa (Hg) ve nikel (Ni) varlığı açısından incelendi. Klinik muayenede sırasıyla 20 ve 13 hayvanda, rumen asidozu ve ovaryum hastalıkları en yaygın hastalıklar olarak tespit edildi. Karaciğer numunelerinin hiçbirinde Ni bulunmadı. Sırasıyla, Al 4.60±8.71 ppm, As 0.39±0.11 ppm, Hg 0.41±0.29 ppm ve Pb 0.04±0.13 ppm olarak tespit edildi. Yaygınlık yüzdeleri toplam hayvan sayısına göre hesaplandı: Al %72, As %100, Hg %88, Ni %0 ve Pb %10. Çalışmada ortalama As değerinin toksik aralıkta olduğu görülmüştür. Al numunelerin %28'i hayvan sağlığı açısından kabul edilebilir limitlerin üzerinde tespit edilmiştir. Hg ortalama değerleri ise insan sağlığı için kabul edilebilir limitlerin üzerindedir. Özet olarak, toksik ağır metaller Al, As ve Hg Holstein karaciğerlerindeki prevalansı oldukça yüksek bulunmuştur.
Anahtar Kelimeler: *Ağır metal, Alüminyum, Arsenik, Civa, Karaciğer, Kurşun.*

Introduction

Nowadays, all living organisms and their environment are constantly exposed to environmental pollutants. Industrial activities are increasing in parallel with the growing population, and air, soil, and water pollution are threatening life on Earth (Bilge and Cimrin, 2013; Kodrik et al., 2011). Heavy metals are dangerous agents that cause significant health problems in animals due to their toxicity. Many industrial areas, fertilizers, traffic, cars, chemicals, groundwater, and animal feeds are substantial sources of heavy metal contamination (Anjulo and Mersso, 2015). The organism absorbs heavy metals through the mouth, respiration, and skin, and most of them cannot be excreted through the body's excretory pathways (kidney, liver, intestine, lung, skin) without special assistance. Therefore, a large portion of heavy metals accumulates in biological organisms. As a result of accumulation, these metals, which concentrate on living organisms, can cause serious diseases (such as thyroid, neurological, autism, and infertility) and even death when they reach effective doses (Özbolat and Tuli, 2016). The liver is a vital storage organ for trace elements and heavy metals. Therefore, the concentrations of these elements in the liver reflect the liver's exposure to these substances (Suttle, 2010). While heavy metal toxicities such as arsenic (As), cadmium (Cd), and lead (Pb) are well known, others like zinc (Zn), copper (Cu), cobalt (Co), manganese (Mn), iron (Fe), magnesium (Mg), and selenium (Se) are necessary in trace amounts for basic physiological functions (Dutta et al., 2022; Pandey et al., 2014; Samy et al., 2022; Sevostyanova et al., 2020). Pb, Cd, As, and mercury (Hg) elements have no defined biological functions and are considered undesirable and potentially toxic contaminants in animal feed (Anjulo and Mersso, 2015; Underwood and Mertz, 1987), and due to their high toxicity, they pose a threat to public health even at very low exposure levels (Tchounwou et al., 2012). The excessive levels of these elements in cattle are generally caused by anthropogenic emissions and food contamination (Alonso, 2012). Pb and Cd negatively affect many biochemical and physiological processes when exposed to sub-lethal doses (Elarabany and El-Batrawy, 2019; Wang et al., 2022). Long-term exposure to heavy metals (Cd, Ni, As, Pb) can affect central nervous system functions and damage the kidneys, lungs, and liver. Among all metals, Pb, As, and Cd have more adverse effects on animal and human health (Patwa et al., 2022; Penticoff and Fortin, 2023). The absence of a reference Veterinary Poison Control Center reporting animal poisoning cases in Türkiye and the European Union makes it difficult to obtain information about clinical poisoning cases (Guitart et al., 2010).

Products used as animal feed may contain undesirable substances that may jeopardize animal health or pose a risk to human health or the environment due to their presence in animal products. It is impossible to avoid undesirable substances altogether, but reducing their quantity in animal feed products is essential. This way, unwanted and harmful effects such as acute toxicity, bioaccumulation, and biodegradability can be prevented. Suppose the products used as animal feed are reliable, free from adulteration, of marketable quality, and used correctly. In that case, they do not pose a risk to human, animal, or environmental health and do not harm livestock production. The presence of certain undesirable substances in complementary feedingstuffs should be limited by setting appropriate maximum levels (EUP Council Directive, 2002).

While there are many scientific studies on the presence of heavy metals in Türkiye, there are only a limited number of studies on the presence of heavy metals in the liver tissue of dairy cows. In the present study, we aimed to investigate the accumulation of heavy metals in the liver of slaughtered dairy Holsteins from the Muğla region and to discuss the potential effects of this accumulation on the health of dairy cows.

Materials and Methods

Ethical Consideration: The present study was approved by the Animal Experiments Local Ethics Committee of Muğla Sıtkı Koçman University (MUDEM-HADYEK) with the ethical approval number of 23.09.2021, 33/21.

Animal material: 50 Holstein dairy cows aged 3 to 13 years from 24 different villages in the province of Muğla/Türkiye were used. The study material was randomly selected from dairy Holsteins brought to the slaughterhouse from Milas, Menteşe, and Yatağan provinces of Muğla city according to the slaughter order.

Clinical examination: Animal materials were Holstein dairy cows brought to the slaughterhouse due to losing their breeding characteristics because of various diseases. All animals were subjected to detailed clinical examinations before slaughter. As a result of the clinical examination, liver samples were taken from 50 animals that had completed their productive lifespan after slaughter.

Liver material and processing: Immediately after the dairy cows were slaughtered, four (4x10 g) 10-gram liver tissue samples were taken from the *lobus caudatus* of the liver (Alonso et al., 2004) and placed in sealed bags. On each sample bag, the ear number of the animal, the name of the farm, its breed, and its age were written. These samples were transported in +4 °C containers to the laboratory. Liver samples not analyzed on the same day were frozen at -20 °C until analysis time. Liver tissue samples collected in sterile petri dishes were dried for 24 hours at 100 °C. Approximately 0.5 g of samples were examined in the ICP OES device after burning 6 ml of HNO_3 (nitric acid) + 2 ml of H_2O_2 (hydrogen peroxide) in the microwave.

Detection of heavy metal presence in samples: Elemental analysis was performed using an ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometer) device. The ICP-OES technique determines the elements in an aqueous solution based on the various wavelengths formed according to the optical properties of the light passed through the plasma. Samples were analyzed for the presence of As, Al, Hg, Ni, and Pb.

Statistical method: Using SPSS 13.0, one-way analysis of variance (ANOVA) was used to evaluate the amount of heavy metals, and the Tukey test was used to determine the source of differences. A value of $p<0.05$ will be considered statistically significant.

Results

Clinical examination: Rumen acidosis and ovarian diseases were identified as the most frequently detected, with 20 and 13 animals, respectively. Other diseases identified during the clinical examination were traumatic reticuloperitonitis, abomasum diseases, omasum constipation, mastitis, and old age, respectively. Clinical examination results are similar to the official health reports. The clinical examination showed no signs of heavy metal toxicity in the animals.

Heavy metals: The study analyzed the presence of 5 different heavy metals in liver samples from 50 Holstein dairy cows. The total and average values and the standard deviation of all heavy metals were presented in Table 1. None of the samples contained Ni. The presence of Pb was only detected in 5 samples, while Hg was found in 44 samples and Al in 36 samples. As was detected in all samples. Concerning the total number of animals, the prevalence was determined as follows: 72% for Al, 100% for As, 88% for Hg, 0% for Ni, and 10% for Pb (Table 2). All heavy metals,

Table 1. Mean and standard deviation and min-max value of heavy metals (dry weight) in the liver of 50 Holsteins.

Element	Mean±SD (ppm)	Min-Max (ppm)
Al	4.60±8.71[a]	0.30-59.52
As	0.39±0.11[b]	0.21-0.65
Hg	0.41±0.29[c]	0.01-1.03
Ni	0±0	0.00-0.00
Pb	0.04±0.13[d]	0.13-0.68

Al: Aluminium, **As:** Arsenic, **Hg:** Mercury, **Ni:** Nickel, **Pb**: Lead, **SD:** Standart deviation, ppm; in original sample mg/kg, a,b, c, d: $p<0.05$ (different letters indicate significant difference)

Table 2. Prevalence of heavy metals in livers of slaughtered dairy Holsteins.

	Aluminum	Arsenic	Mercury	Nickel	Lead
n total	50	50	50	50	50
positive	36	50	44	0	5
negative	14	0	6	100	45
Overall positive (%)	**72.0**	**100.0**	**88.0**	**0.0**	**10.0**
>0.5 ppm positive (%)	**70.0**	**20.0**	**34.0**	**0.0**	**4.0**
>1.0 ppm positive (%)	**60.0**	**0.0**	**2.0**	**0.0**	**0.0**

including Al, As, and Hg, were detected in 32 samples. The total ppm values of heavy metals were measured as Al 229.86 ppm, As 19.65 ppm, Hg 20.74 ppm, Ni 0.00 ppm, and Pb 1.79 ppm. The average value of aluminum was higher compared to other heavy metals. Al was not detected in 14 liver tissues. Al was detected in varying amounts in 36 samples. In the detected tissues, the lowest value of aluminum was measured at 0.30 ppm, while the highest value was 59.92 ppm. As was detected at different levels in all samples. The lowest As value detected was 0.21 ppm, while the highest was 0.65 ppm. Hg was detected at various levels in 44 of the samples. The lowest level of Hg detected was 0.01 ppm, while the highest level detected was 1.03 ppm. Ni was not detected in any of the samples. Pb was not detected in 45 of the samples. Pb was detected in 5 samples, with the lowest value measured at 0.22 ppm and the highest at 0.68 ppm. Among the heavy metals, Al was detected in the most significant quantity at 229.86 ppm. As was detected in every sample, making it the heavy metal with the highest percentage occurrence. Conversely, Ni was the only heavy metal not found in the analysis, as it was absent in all samples. The average amount of As was categorized as hazardous in the study. The amount of Al in 28% of the samples was classified as harmful. Conversely, the average Hg levels are higher than what is considered safe for human health.

Heavy metal accumulations between the districts (Milas, Yatağan, Menteşe) were compared, and no significant difference was found between the districts ($p>0.05$). Animals were divided into groups 1-6 years and 7 years and older. The heavy metal accumulations in cattle were compared according to their ages, but no significant differences were found between the age groups ($p>0.05$)

Additionally, the amounts of heavy metals detected in liver tissues from various studies conducted in Türkiye and worldwide are presented in Table 3.

Discussion and Conclusion

Health problems arising from poisoning in animals appear to be a rare health issue compared to other clinical diseases, such as infectious diseases, trauma, or neoplasia. One reason may be the lack of information about the most common toxins affecting veterinary species, resulting in very little information to guide and facilitate diagnosis. The European Union (EU) does not have a Veterinary Poison Control Center that centralizes and publishes poisoning information, and even outside the European Union, data is often managed by different institutions, mostly Veterinary Medicine or Science faculties, and is limited in scope. This leads to the inadequate and limited availability of toxicological epidemiological data, with very little or no accessibility for veterinarians in other regions or countries. There are no annual reported poisoning case records, but there is region-specific case-based information in studies published in peer-reviewed journals (Guitart et al., 2010).

Table 3. The concentration of heavy metals reported by other studies in liver tissues of cattle.

Heavy metal	n	Dry/wet	Mean or median value (ppm)	Country	References
Pb	172	wet	0.1-1.0	Western Canada	(Cowan and Blakley, 2016)
Pb	56	wet	0.0475	Spain	(Alonso et al., 2000)
Pb	1254	wet	0.07 ± 0.05	Canada	(Salisbury et al., 1991)
Pb	3	wet	16.78	Austria	(Krametter-Froetscher et al., 2007)
Pb	10	dry	0.0079 ± 0.0015	Saudi Arabia	(Meligy et al., 2019)
Pb	120	wet	0.028	Spain	(Alonso et al., 2004)
Pb	180	wet	0.103 ± 0.130	Pakistan	(Mushtaq et al., 2024)
Pb	61	wet	0.12	Brasil	(Aranha et al., 1994)
Pb	87	wet	0.059	Germany	(Kreuzer et al., 1988)
Pb	30	wet	0.405	Italy	(Amodio-Cocchieri and Fiore, 1987)
Pb	290	wet	0.160	Poland	(Falandysz, 1993)
Pb	6	wet	0.465	Slovac Republic	(Kottferova and Koréneková, 1995)
Pb	21	wet	1.072	Slovac Republic	(Koréneková et al., 2002)
Pb	68	wet	0.10	Slovenia	(Doganoc, 1996)
Pb	112	wet	0.047	Zambia	(Yabe et al., 2012)
Pb	90	dry	0.059±0.020	Colombia	(Madero and Marrugo-Negrete, 2011)
Pb	50	dry	0.485	Türkiye	(Kocasari et al., 2016)
Pb	698	dry	0.3	Netherlands	(Counotte et al., 2019)
Pb	30	wet	0.05 ± 0.02	Spain	(Rodríguez-Marín et al., 2019)
Pb	30	wet	0.23 ± 0.26	Spain	(Rodríguez-Marín et al., 2019)
As	56	wet	0.0102	Spain	(Alonso et al., 2000)
As	351	wet	0.03 ± 0.01	Canada	(Salisbury et al., 1991)
As	120	wet	ND	Spain	(Alonso et al., 2004)
As	15	dry	0.14±0.03	Türkiye	(Oymak et al., 2017)
As	10	dry	0.0111 ± 0.025	Saudi Arabia	(Meligy et al., 2019)
As	156	dry	3.2-350	in the World	(Bertin et al., 2013)
As	180	wet	0.333 ± 0.200	Pakistan	(Mushtaq et al., 2024)
As	112	wet	0.002	Zambia	(Yabe et al., 2012)
As	571	wet	0.01	Norway	(Kluge-Berge et al., 1992)
As	50	dry	ND	Türkiye	(Kocasari et al., 2016)
Hg	624	wet	0.01 ±0.01	Canada	(Salisbury et al., 1991)
Hg	146	wet	0.003	Netherlands	(Vos et al., 1987)
Hg	112	wet	0.0003	Zambia	(Yabe et al., 2012)
Hg	3	wet	0.873/0.750/ <0.001	Austria	(Krametter-Froetscher et al., 2007)
Hg	120	wet	ND	Spain	(Alonso et al., 2004)
Hg	114	wet	<0.010-0.012	Finland	(Niemi et al., 1981)
Hg	340	wet	0.00765-0.00101	Spain	(Alonso et al., 2003)
Hg	1036	wet	0.002 ± 0.0022	Poland	(Nawrocka et al., 2020)
Hg	1096	wet	0.008	Poland	(Szprengier-Juszkiewicz, 1994)
Hg	33	wet	0.047	Sweden	(Jorhem et al., 1991)
Hg	470	wet	0.006	Poland	(Zmudzki et al., 1991)
Hg	72	wet	0.002	Iran	(Hashemi, 2018)
Hg	6	wet	0.0243	Czech Republic	(Čelechovská, 2008)
Hg	6	wet	0.0033	Czech Republic	(Čelechovská, 2008)
Hg	180	wet	0.425 ± 1.110	Pakistan	(Mushtaq et al., 2024)
Hg	90	dry	0.028±0.025	Colombia	(Madero and Marrugo-Negrete, 2011)
Al	15	dry	2.44±1.06	Türkiye	(Oymak et al., 2017)
Al	30	wet	55.3 ± 58.0	Spain	(Rodríguez-Marín et al., 2019)
Al	30	wet	8.65 ± 4.38	Spain	(Rodríguez-Marín et al., 2019)
Ni	112	wet	0.594	Zambia	(Yabe et al., 2012)
Ni	21	wet	0.231	Slovac Republic	(Koréneková et al., 2002)
Ni	694	dry	0.3	Netherlands	(Counotte et al., 2019)
Ni	120	wet	ND	Spain	(Alonso et al., 2004)

ND: not detected, **Pb:** lead, **As:** arsenic, **Hg:** mercury, **Al:** aluminum, **Ni:** nickel: **n:** animals number

The acute lethal oral dose for Pb is 200 to 400 mg/kg body weight (bw) for calves and 600 to 800 mg/kg bw for adult cattle (Payne and Livesey, 2010). The toxic dose of Pb for the liver is reported to be 33.5 mg/kg (Counotte et al., 2019; Cowan and Blakley, 2016). Radostits et al. (2002) reported that the toxic dose of Pb in the liver of ruminants is between 10 and 20 ppm, and the toxic dose in the kidneys is 20 ppm. In another study, an acute toxicity value of over 10 ppm for Pb in the kidneys and liver was reported (Baker, 1987). Amodio-Cocchieri and Fiore (1987) and Cowan and Blakley (2016) reported the normal values in liver tissue as 0.405±0.365 and 0.1-1.0 ppm, respectively.

In the current study, the highest level detected in the liver was 0.68 ppm, which is not toxic for a cow. In our study, Pb was detected in the liver of only four animals, which are below harmful levels.

Barceloux (1999) reported very little evidence that Ni compounds accumulate in the food chain and that Ni is not a cumulative toxin in animals or humans. In line with this information, the absence of Ni in any of our samples supports Barceloux's views.

In a study conducted by Counotte et al. (2019) in the Netherlands between 2007 and 2018, 1544 cattle livers were examined and toxic levels of Pb were only observed in the youngest group of cattle. Cowan and Blakley (2016) categorise the age groups in 525 cases of acute Pb poisoning that occurred in northern Canada between 1998 and 2013, and no significant difference is found. In their study, Şimşek and Dinçel (2023) revealed that the concentrations of certain heavy metals change with age and that heavy metal accumulation is higher in adult cattle. The current study did not determine the relationship between heavy metal accumulation and age. In our study, no significant difference was found between age groups ($p>0.05$).

In acute toxicosis, Hg residues in the kidney exceed 10–15 ppm (Buck, 1975), while in the liver, they exceed 100 ppm (Hapke, 1988). In our study, the values detected as an average of 0.41 ppm and a maximum of 1.03 ppm are below the toxic dose for cattle. However, it has been detected above the tolerable level for human health, 0.1 ppm, as determined by the Austrian Ministry of Health. At the same time, in the Netherlands, the maximum acceptable concentration for human health in bovine liver tissue is 0.05 ppm (Vos et al., 1987). In line with these values, the values we obtained are seen to be above the acceptable levels for human health. In the study conducted by Alonso et al. (2003) in Spain, Hg was detected in the liver of 19.6% of 56 cattle. In contrast, in our study, different amounts of Hg were detected in 88% of them. This result is not consistent with our study. In another study conducted in Poland, various amounts of Hg were detected in 69% of 1036 cattle (Nawrocka et al., 2020). The results of this study appear to be more consistent with our study. In a study conducted on the liver of beef cattle in Colombia, the values of Hg and Pb were determined to be 0.028±0.025 and 0.059±0.020 ppm, respectively. The results were below the detected values for human and animal health (Madero and Marrugo-Negrete, 2011). The Pb value is consistent with the average value in our study, while the Hg value was much lower than the value we detected. In a study conducted on 180 cattle livers in Pakistan, levels of 0.333±0.200 ppm As, 0.103±0.130 ppm Pb, and 0.425±1.110 ppm Hg were detected, respectively (Mushtaq et al., 2024). As and Hg values are close to those in our study, while the Pb value is observed to be at a higher level than our study. In another study conducted on 50 cattle livers in Türkiye, As was detected in only one of the samples, and Hg was not detected in any of the samples. Pb was detected in 2 samples (Kocasari et al., 2016) in the current study, As was detected in all samples, Hg in 44 samples, and Pb in 5 samples.

Reference ranges for As, obtained from the Diagnostic Center for Population and Animal Health at Michigan State University, are 0.05–0.17 ppm in urine, <50 ppb in blood, 0.004–0.40 ppm in liver, and 0.018–0.40 ppm in kidney (Bertin et al., 2013). Animals can tolerate low levels of As; the normal level in cattle tissues is approximately 0.5 ppm. When As level in the liver exceeds 10 to 15 ppm and is accompanied by clinical symptoms, acute As poisoning is diagnostically considered (Bahri and Romdane, 1991; Fletcher, 1986; Monies, 1999). In humans, the toxic dose is 10-50 mg, and the lethal dose (LD50: lethal dose) is 60-200 mg (Aliyev, 2011; Dousova et al., 2003; Or, 1996). The As levels detected in the current study are not toxic to either animal or human health. According to Chopra et al. (2017), cattle are more vulnerable to As poisoning than other species. Bertin et al. (2013) compiled cases of As poisoning published in peer-reviewed journals between 1941 and 2012 in a study and identified 156 cases. This result indicates that As poisoning is not very common or that the studies are insufficient. A study conducted on 10 cattle livers in Saudi Arabia detected 0.0079 ppm Pb and 0.0111 ppm As levels (Meligy et al., 2019). According to our study, the detected values are pretty low. In a study conducted in Türkiye, As was detected in only 1 out of 50 cattle livers, while none of the samples contained Hg (Kocasari et al., 2016). In the current study, As was detected in all 50 samples, while Hg was detected in 44 samples

In cattle and sheep, aluminum concentrations of 6-11 ppm in the liver and 4-5 ppm in the kidney are considered toxic. A levels ≥1.2 ppm in dog liver are considered high (Gupta, 2012). In the current study, the average was 4.60 ppm, below the toxic level. In comparison, 14 animals had liver Al levels detected at six ppm and above, indicating they were within the toxic dose range. A very high level of 59.92 ppm was detected in only one animal. In a study conducted on 15 cattle in Türkiye in 2017, the average Al was determined to be 2.44±1.06 ppm, and the average As was determined to be 0.14±0.03 ppm (Oymak et al., 2017). The average values of Al and As were lower in our study.

In conclusion, the prevalence of the toxic heavy metals Al, As and Hg in the liver of Holsteins was high. Al and Hg levels in the liver can be categorised as hazardous to animal and human health even at trace levels. Toxic heavy metals, even at minimal levels, pose significant potential dangers to animal and human health. Animal feeds, waters, soil, and industrial areas can be serious sources of contamination for heavy metals. In this regard, it is very important to regularly identify the damage caused by relevant environmental contaminant risks to the health of animals and humans.

Conflict of Interest

Authors declare no conflicts of interest.

Ethical Approval

This study protocol was approved by the Ethics Committee of Muğla Sıtkı Koçman University with approval number (MUDEM-HADYEK), 23.09.2021, 33/21.

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