Ankara Univ Vet Fak Derg, **69**, 83-89, 2022
DOI: 10.33988/auvfd.835745

IPARD supports positively affect hematological parameters, milk production, and welfare indicators of dairy cows

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Received date: 04.12.2020 - Accepted date: 30.04.2021

Abstract: The study investigated body condition score (BCS), hematological parameters, metabolic profile indicators, total oxidant-antioxidant status, and milk yield (MY) of cows in non-funded (NF) and funded (F+) dairy farms by the European Union funds (under the Instrument for Pre-accession Assistance for Rural Development-IPARD) to support rural development. Sixty multiparous Holstein cows (110 to 209 days of lactation) from 3 NF and 3 F+ dairy farms were used in the experiment. Hematological parameters were within physiological limits in both dairy farms. Total erythrocyte count and percent hematocrit were higher in the F+ group. Serum non-esterified fatty acids (NEFA) level and aspartate aminotransferase (AST) activity were higher in NF farms compared to the supported ones. The serum triglyceride, total cholesterol, glucose levels, and gamma-glutamyl transferase (GGT) activity were higher for the cows in F+ farms. The average daily MY and BCS of cows in NF and F+ dairy farms were 15.8 L and 2.83, and 24.4 L and 3.11, respectively ($P<0.01$). The total antioxidant status (TAS) of cows in NF farms was significantly lower than F+ farms, whereas total oxidant status (TOS) was significantly higher for cows in NF farms. In conclusion, the grant support is important in establishing a more capable, more efficient, and more suitable infrastructure for animal welfare, and positively affects the physiological health and efficiency of animals in these dairy farms.
Keywords: Animal welfare, body condition score, metabolic profile, physiological parameters, oxidant-antioxidant status.

IPARD destekleri süt ineklerinde hematolojik parametreleri, süt verimini ve hayvan refah göstergelerini olumlu etkilemektedir

Özet: Çalışma, kırsal kalkınmayı desteklemek için Avrupa Birliği fonları tarafından verilen "Kırsal Kalkınma İçin Katılım Öncesi Yardım Aracı-IPARD" kapsamındaki hibelerin süt ineklerinde vücut kondüsyon skoru (VKS), hematolojik parametreleri, metabolik profil göstergeleri, toplam oksidan-antioksidan durumu ve süt verimine etkilerini araştırmayı hedeflemektedir. Bu çalışmada IPARD desteği alan (F+) üç çiftlik ve destek almamış (NF) üç çiftlikte 110-209 günler arası laktasyon periyodundaki toplam 60 multipar Holstein ineği rastgele seçildi. Bulgulara göre, hem NF hem de F+ çiftliklerindeki ineklerin hematolojik parametreleri fizyolojik sınırlar içerisindedir. Ancak NF grubunda F+ grubuna göre serum NEFA düzeyi ve AST aktivitesi daha yüksek, F+ grubu ineklerin ise serum trigliserid, toplam kolesterol, glikoz seviyeleri ve GGT aktivitesi, eritrosit ve hematokrit seviyeleri NF grubuna göre daha yüksek bulunmuştur. NF grubunda günlük ortalama süt verimi ve VKS değerleri 15,8 L ve 2,83, F+ grubunu oluşturan süt çiftliklerinde ise 24,4 L ve 3,11'dir ($P<0,01$). NF çiftliklerinde ineklerin toplam antioksidan durumu F+ çiftliklerindeki ineklerden önemli ölçüde düşük, önemli bir risk faktörü olan toplam oksidan statü ise önemli ölçüde yüksekti. Sonuç olarak, hibe desteğinin hayvanlarda fizyolojik göstergeleri olumlu olarak etkilediği, bu hayvanların barınma, bakım ve besleme koşullarının sürekli kontrol ediliyor olması nedeniyle hayvan refahına daha uygun olduğu izlenmiştir. Hayvanların daha uygun ve izlenebilir bir altyapıya sahip olmaları açısından IPARD hibe desteklerinin sürdürülebilir hayvan sağlığı ve verimliliği için önemli olduğu düşünülmüştür. Sonuç olarak bu süt çiftliklerindeki hayvanların fizyolojik sağlığı, kondüsyonları ve verimliliklerinin IPARD desteklerinin sağladığı koşullardan olumlu yönde etkilendiği görülmüştür.
Anahtar sözcükler: Fizyolojik göstergeler, hayvan refahı, metabolik profil, oksidan-antioksidan statü, vücut kondüsyon skoru.

Introduction

The supports for rural development have increased significantly in the last couple of decades and several support programs are in effect. The main objectives for such support programs are protecting and developing genetic resources at their original places, updating the recording systems, improving farming and healthier production, ensuring sustainability, increasing the effectiveness of farming policies, preventing animal diseases, and supporting animal health and welfare (33).

A fast and accurate instrument to understand the welfare, physiological status and productivity of animal are not only important for businesses and researchers but it is also significant for the country's economy and global animal husbandry research. Recently, efficient, safe, and sustainable agriculture and livestock practices have become the common agenda of the international community. Animal health and welfare should be prioritized for activities with these qualifications (7). For this reason, increasing and updating the quality standards of animal life and animal products has become a basic agricultural policy, and has increased in the agenda of the researchers. It is generally accepted in the international community that the most appropriate step in this area is to support rural development (2). One of these support programs was founded by the European Union to aid candidate and/or potential candidate countries by the IPA Council Regulation (EC) No 1085/2006, the Instrument for Pre-Accession Assistance (IPA). This support has different components and Turkey as a candidate country has the right to benefit from all components (8, 33). Thus, the Instrument for Pre-Accession Rural Development (IPARD) grant support program was started to implement as IPARD-I for the first time in Turkey, covering the years 2007-2013 (17). Considering that 87% of the enterprises in Turkey are small businesses, the contribution of this grant support to production and quality to these agricultural enterprises is very important (33). Thus, this study was carried out to compare funded (F+) and non-funded (NF) small-medium sized dairy cattle enterprises that were established within the scope of the IPARD-I program during 2007-2013 in Afyonkarahisar province. For this purpose, hematological parameters, metabolic indicators, oxidant-antioxidant status, body condition score (BCS), and milk yield (MY) characteristics of cows in F+ and NF dairy farms were evaluated during the experiment.

Materials and Methods

The research was carried out with the approval of Afyon Kocatepe University, Experimental Animals Ethics Committee (AKÜHADYEK - 467-15). Data from 60 Holstein cows at 110-209 days of lactation (2 to 5 years old) were studied in the experiment. The cows were randomly selected from the six dairy farms [three IPARD funded (F+, n=30) and three non-funded (NF, n=30) dairy farms]. All dairy farms were the members of the Breeders Association in the Afyonkarahisar region. The median days in lactation for the cows in F+ and NF dairy farms were 145 and 156 days, respectively. The median number of lactation was 3 in both groups. The rations given to cows during our study period were in Table 1. MY values were acquired from the individual farm records.

Table 1. Rations used in IPARD supported (F+) and not supported (NF) dairy farms.

Nutrients	F+	NF
Dry Matter, kg/day	28.10	24.19
NEL, Mcal/day	41.58	35.55
Crude Protein, %	15.35	13.18
Rumen Degradable Protein, %	36.01	58.44
Bypass Protein, %	63.99	41.56
NDF, %	36.69	41.67
ADF, %	22.16	26.24
NFC, %	38.39	36.30
Fat, %	4.50	3.78
Ca, %	0.92	0.93
P, %	0.39	0.32

NEL=Net energy for lactation, NDF=Neutral detergent fiber, ADF=Acid detergent fiber, NFC= Nonstructural carbohydrates.

General characteristics of the farms: All F+ farms had more than 60 milking cows, the udders were cleaned before and after the milkings, and milking was performed twice a day with an automated milking system. On the other hand, the NF farms had 18 to 25 milking cows. These farms had mobile milking machines, the udders were cleaned usually only before (50%) or after (30%) the milkings and the milking frequencies were twice a day. The cows in F+ farms received regular veterinary service, the manure was removed regularly from the stalls by automated scrapers, and the manure was stored in storage facilities. The hoof care was performed once every six months. The cows in NF farms mostly received veterinary service when there was a major health problem, the manure was collected manually and transferred to the field or stored next to the barns, and hoof care was performed once a year.

Blood samples and analyses: Vena jugularis was used for blood collection and the collection procedure was performed between 06:00 and 07:00 am before the morning feeding or milking. The blood was collected directly into anticoagulant free and EDTA coated vacutainer tubes (Vacutest, Arzergrande, Italy). In the EDTA coated tubes, hematological parameters were measured by using an auto-analyzer (Mindray BC-2800

Vet blood count, Shenzhen, China). The remaining blood samples and other anticoagulant free tubes were centrifuged at 4 ºC for 10 minutes (NF 1000R, at 1500xg). Serum and plasma samples were kept at -20 ºC until used for total antioxidant status (TAS), total oxidant status (TOS), and metabolic profile analyzes.

A fully automated ELISA reader (Chemwell 2910, Awareness Tech. Inc., USA) was used for the analysis of metabolic and biochemical parameters from the serum samples. While serum non-esterified fatty acid (NEFA) and beta-hydroxybutyrate (BHBA) values were measured using species-specific bovine kits (Randox Laboratories®, Crumlin, UK), glucose (GLU), total cholesterol (CHOL), triglyceride (TG) levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT) activities were determined using species-nonspecific kits (Biolabo SA®, France). Total antioxidant status and TOS levels were measured in serum samples of dairy cows with the specific ELISA kit (Assay Rel Diagnostics, Turkey).

Body condition scoring: Body condition scoring was performed according to the guidelines described by Edmonson et al. before the morning feeding or milking (11). The amount of fat covering the tail head, vertebrae, loin, and rump were evaluated with a score from thin to fat (1 to 5; 1 referred to extreme emaciation and 5 referred to obese; in increments of 0.25). The same individual judge evaluated the cows. The most critical areas evaluated were vertebrae at the middle of the back, rear view of the hook bones, side view of the line between the hook and pin bones, and a cavity between the tail head and pin bones.

Statistical analysis: The data obtained in the study were evaluated using the SPSS 17.0 statistical program. First, the normality tests of the data were performed for the farms that constitute F+ and NF groups. Independent two samples t-test procedure was used for comparison of F+ and NF farms. For statistical significance, $P<0.05$ value was chosen and values were expressed as mean ± standard deviation.

Results

In general, the parameters tested in the current study were homogeneous for the farms that constitute F+ and NF groups. In addition, there was no statistical difference among the 3 farms that represent the F+ group, and the 3 farms that represent the NF group for hematological parameters, biochemical and oxidant-antioxidant indicators, BCS, or MY.

Comparisons of the hematological parameters such as red blood cell counts (RBC), hematocrit (HCT), hemoglobin (HMG), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and platelet counts (PLT) between F+ and NF dairy farms were in Table 2. There was a statistically significant difference between F+ and NF farms in terms of RBC ($P<0.05$). Although HMG and MCV did not significantly differ, HCT ($P<0.05$) and MCH ($P<0.05$) were different between F+ and NF farms (Table 2). Moreover, PLT ($P<0.001$) was higher for cows in F+ farms (Table 2).

Table 2. Hematological parameters of dairy cows in 3 IPARD supported (F+, n=30) and 3 not supported (NF, n=30) dairy farms.

Parameters	NF	F+	P
RBC (10^{12}/L)	6.14 ± 0.70	6.60 ± 0.91	0.02
HMG (g/dL)	10.0 ± 1.03	10.3 ± 0.92	NS
HCT (%)	27.5 ± 3.39	30.1 ± 2.64	0.002
MCV (fL)	44.8 ± 3.29	45.7 ± 4.85	NS
MCH (pg)	16.4 ± 1.15	15.7 ± 1.60	0.05
MCHC (g/dL)	22.5 ± 2.34	22.9 ± 3.19	NS
WBC (10^9/L)	8.6 ± 2.60	10.3 ± 4.37	NS
PLT (10^9/L)	296.4 ± 143.1	438.5 ± 109.9	0.001

RBC = red blood cell, HMG = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, WBC = white blood cell, PLT = platelet, NS = not significant.

The data on biochemical indicators such as AST, ALT, and GGT activities, BHBA, GLU, CHOL, and TG levels for F+ and NF groups were shown in Table 3. Although serum ALT activity, BHBA and BUN concentrations did not differ significantly, considerable alterations were apparent for serum activities of AST ($P<0.001$), GGT ($P<0.05$), NEFA ($P<0.001$), GLU ($P<0.001$), CHOL ($P<0.01$), and TG ($P<0.001$) levels between the F+ and NF groups (Table 3).

Table 3. Biochemical parameters of dairy cows in 3 IPARD supported (F+, n=30) and 3 not supported (NF, n=30) dairy farms

Parameters	NF	F+	P
BHBA (mmol)	0.43 ± 0.34	0.36 ± 0.12	NS
NEFA (mmol)	0.85 ± 0.62	0.22 ± 0.18	0.001
AST (U/L)	111.3 ± 18.0	92.20 ± 7.97	0.001
ALT (U/L)	26.0 ± 7.44	27.9 ± 8.60	NS
GGT (U/L)	22.4 ± 8.34	28.0 ± 7.73	0.02
GLU (mg/dL)	52.8 ± 2.98	58.5 ± 4.25	0.001
BUN (mg/dL)	17.9 ± 4.59	20.1 ± 6.47	NS
CHOL (mg/dL)	129.0 ± 28.1	157.5 ± 40.1	0.003
TG (mg/dL)	15.2 ± 4.13	19.4 ± 3.71	0.001

BHBA = beta hydroxy butyrate, NEFA = non-esterified fatty acid, AST = Aspartate Aminotransferase, ALT = Alanine Aminotransferase, ALP = Alkaline Phosphatase, GGT = Gama Glutamyl Transferase, GLU = glucose, BUN = blood urea nitrogen, CHOL = cholesterol, TG = triglyceride, NS = not significant.

Table 4. Oxidant-antioxidant status, body condition score (BCS) and milk yield (MY) of dairy cows in 3 IPARD supported (F+, n=30) and 3 not supported (NF, n=30) dairy farms.

Parameters	F+	NF	P
TAS (μmol Trolox equivalent/L)	0.98 ± 0.33	0.53 ± 0.45	0.001
TOS (μmol H_20_2 equivalent/L)	1.50 ± 1.41	3.16 ± 1.35	0.001
BCS (1 to 5)	3.11 ± 0.41	2.83 ± 0.47	0.01
MY (kg)	24.4 ± 5.18	15.8 ± 3.23	0.01

TAS = total antioxidant status, TOS = total oxidant status.

The oxidant-antioxidant indicators and BCS-MY values are in Table 4. As an indicator of antioxidant status, TAS values were higher in F+ dairy farms compared to NF dairy farms (P<0.001). On the contrary, TOS values were significantly lower for cows in the F+ group (P<0.001; Table 4). In addition, both BCS and MY values were significantly higher in the F+ group than the NF group (P<0.01).

Discussion and Conclusion

There are several studies on the biochemical and hematological indicators, antioxidant-oxidant status, MY, and BCS status of dairy cows in different lactation periods (5, 22, 31). However, studies on how IPARD support might affect biochemical and hematological indicators, antioxidant-oxidant status, MY, and BCS of cows in dairy enterprises are not available in the current literature. It is important to discuss the indicators and productivity of animals in F+ and NF dairy farms, considering that the grant-supported farms are better equipped and have a higher concern for animal health and well-being (33). It has been observed that the F+ farms sampled in the current study had a better environment for their cows compared to NF. The higher consideration for animal health and well-being of the cows in the F+ farms were generally reflected in hematological profiles, biochemical indicators, oxidant-antioxidant status, BCS scores, and MY values.

Avidar et al. (3) and Rowlands (24) established a link between the hematological and biochemical profiles of animals and their milk production. In the current study, RBC, platelet, and HCT values were higher in the F+ group. It is known that blood cells may vary depending on factors such as age, race, gender, geographical altitude, and environment. Moreover, animals living in better conditions tended to have higher blood parameters (35). Considering that the cows used in the current study were in the same race, gender, altitude, and similar geographical conditions, it can be said that the improved hematological values for dairy cows in F+ farms might be positively affected by the care and feeding conditions. In addition, it was evaluated that the blood cells may be increased due to the increased metabolic and immunological needs of animals with higher MY. Apart from our study, no study investigating hematological indicators of dairy cows in IPARD supported farms has been encountered. In our study, the levels of RBC were within physiological limits in both F+ and NF groups. However, the higher RBC levels for the cows in the F+ group supports increased oxygen-carrying and utilization capacity and therefore the metabolic activity for these cows which, in turn, augments efficiency (30).

The serum CHOL level is an important indicator because it gives information about lipid metabolism (34). In our study, when F+ and NF groups were compared, the CHOL level was significantly higher for cows in the F+ group. Different studies stated that serum CHOL levels were affected by MY at different lactation stages. Rowlands (24) also suggested that there was a link between MY and serum CHOL. There was a positive relationship between serum CHOL level and MY after calving and serum CHOL levels increased with the advancement of lactation (23, 26). In another study, Gueorguieva (15) emphasized that there was an increase in CHOL levels, especially in the early lactation phase, so there was a positive correlation between MY and serum CHOL levels.

The serum TG level may be an important marker for dairy cows, especially during the transition period and it is an important parameter that gives information about liver fat metabolism (16). Turgut (29) stated that serum TG level should be in the reference range of 0 to14 mg/dL. In the current study, the serum TG levels for the cows in the F+ group were slightly above this reference value, and also higher than for the cows in the NF group. The elevation seen in serum TG levels in the F+ group might be related to higher metabolism and MY. Sevinç and Aslan (27) stated that serum CHOL and TG levels declined not before, but during the fatty liver syndrome in dairy cows. High-yielding dairy cattle are at risk of the fatty liver syndrome. Relatively high levels of serum TG can be an important buffer against fatty liver syndrome when the levels were within the reference range (27). Thus, relatively higher serum TG levels for cows in the F+ group could be a positive factor that protects them against fatty liver.

Fatty liver is an important metabolic disorder, mostly seen postpartum. Serum NEFA and BHBA levels are important parameters that give vital information about body fat mobilization level, the status of carbohydrate metabolism, and some metabolic disorders such as ketosis and fatty liver (14). It has been emphasized that there will be an increase in serum BHBA and NEFA levels in dairy cows following parturition, but cows may find it difficult to balance the energy input and output after calving (13). Serum NEFA and BHBA can give information about the dairy cows' energy metabolism during the transition period through mid-lactation. Accordingly, the elevated serum levels of these two parameters are good indicators that animals are fed inadequately (14). Consequently, the improved biochemical parameters, such as CHOL, TG, BHBA, and NEFA support the conclusion that the monitoring processes, better rations, and higher standards provided by IPARD support were beneficial to protect animal health and physiology in the supported farms.

When energy intake is limited, it causes excessive fatty acid mobilization to support milk production. Increased serum levels of NEFA can lead to an increased NEFA uptake and TG storage by the liver. Thus, postpartum high serum levels of NEFA can cause fatty liver (14). In the current study, serum NEFA levels were higher in NF farms than F+ farms. This suggests that dairy cows in F+ were less affected by the negative energy balance expected after calving and these cows overcame the harmful effects of negative energy balance in a shorter period when compared to the cows in the NF farm.

The physiological status of the liver can be determined by looking at the serum levels of metabolites produced in the liver. For example, during the breakdown of liver cells, ALT enters the blood. When there is damage to liver tissues, the amount of ALT in the blood increases. Therefore, ALT is often used in detecting liver damage. Serum AST levels, on the other hand, increase during the cellular damage in the RBC or tissue damage in the liver, heart, skeletal muscles, pancreas, and kidneys (28). In dairy cows, serum ALT and AST ranges were reported as 14-38 and as 78-132 IU/L, respectively (9). There was a correlation between serum ALT-AST activities and milk production (3, 24), and the serum ALT and AST activities increased in lactating dairy cows (18). In the current study, the serum activities of ALT and AST in both groups were within normal physiological limits, suggesting that the related tissues such as liver, heart, skeletal muscle, pancreas, and kidneys were in healthy conditions.

Kweon et al. (21) stated that oxidant-antioxidant status affects vital metabolism. Furthermore, oxidative stress is one of the primary risks for physiological processes and provides a predisposition for many diseases (5). It was emphasized that cows with high MY tend to have oxidative stress and it can be an important risk factor for dairy cows during early and mid-lactation (4, 5, 9). As a result, antioxidant additives may affect lactation period performance in dairy cows (9). The sum of oxidative indicators is generally measured and determined by TOS levels (10). In the current study, higher TOS and lower TAS levels for cows in NF farms imply that the oxidative stress at calving probably continued through the mid-lactation in this group at a higher level. Accordingly, the lower TOS and higher TAS values for the cows in the F+ farms suggest that there were fewer oxidative factors present after calving. Therefore, improved environmental factors such as noise, dust and contamination, and better welfare could lower the stress, improve TAS and TOS levels, and support the antioxidant status of the cows in F+ farms.

Body condition scoring is one of the indicators that have a direct effect on MY and fertility rates of dairy cows. Although live weight can vary from cow to cow, BCS should be comparable and within the ideal limits in a given lactation period since ideal BCS is an important criterion in determining the energy and nutritional needs of dairy cows (12). Thus, BSC is an important tool to predict whether animals are being fed properly and BCS monitoring is very important in cases where MY increases (12). When the ideal BCS cannot be achieved, infertility problems are usually experienced in over and underweight cows (6, 31). In the current study, the BCS of F+ and NF farms were 3.11 and 2.83, respectively. Overall, studies usually stated that a BCS of 3.0 to 3.5 is ideal in the mid-lactation period for dairy cows (20). Compared to the previous studies, the average BCS of cows in NF farms fell below the ideal BCS of 3.0, whereas the BCS of cows in F+ farms were in the desired range. Losses can occur in BCS when dairy cows cannot meet their energy needs. BCS is an important follow-up tool on animal welfare and cows should be fed with well-balanced rations to prevent the loss in BCS (32). In the current study, the ration content and energy-protein balance were better in F+ than in NF farms. Moreover, it seems that the IPARD supports helped to improve physical, managerial, and psychological conditions in the F+ farms and this could explain the better BCS in F+ farms.

Paralleled with BCS, MY was also affected by IPARD support. There was a difference in MY between F+ and NF dairy farms. While the mean daily MY of cows in NF farms was 15.0 L, that of cows in F+ farms was 24.4 L. Therefore, MY in dairy farms that were supported by IPARD was significantly higher. Alpan and Arpacık (1) stated that MY in dairy cows can be affected by hereditary and environmental factors. Nutritional factors are also effective in MY (19, 25). Better environmental factors and nutritional status of cows in F+ dairy farms could explain the higher MY and better BCS for the cows in these farms. Moreover, better animal care and the environment could also decrease the possible metabolic disorders. Thus, it is expected that the daily MY productions of IPARD supported farms are likely to be high because nutritional imbalances and metabolic disorders are known to affect MY (35).

Overall, the farms supported by IPARD had better animal care and feeding systems, the sensitivity of farmworkers was higher, animal health and welfare was monitored better, and these farms had more modern equipment. The improved conditions could help to minimize stress in the farms supported by IPARD. In addition, the fact that these farms were checked periodically by the Agricultural and Rural Development Support Institution experts and these regular controls could have a positive impact on the farm management to act more scientifically. In this sense, the health practices, animal care and feeding conditions, and farming equipment in family-owned medium-sized enterprises were far behind compared to IPARD-supported farms.

In conclusion, IPARD grant support positively affected the physiological processes, yield qualities, and animal welfare of the dairy cows because of its positive contributions to the maintenance, care, feeding, and animal material, which provided important contributions to the national economy and global economic processes as well as human, animal and environmental health. Considering that IPARD grants can possibly lead to creating sustainable systems for animal health-welfare, as well as safer and stable products, this support program can be recommended to small and medium-sized businesses in countries with high potential to improve their agricultural production.

Acknowledgements

The current study is produced from a PhD thesis supported by Afyon Kocatepe University. The authors would like to thank the farmers for their participation in the study.

Financial Support

Financial support for this research was obtained from Afyon Kocatepe University, Scientific Research Projects Commission, with Project number 15.SAĞ.BİL.27.

Ethical Statement

The research was carried out with the approval of Afyon Kocatepe University, Experimental Animals Ethics Committee (AKÜHADYEK - 467-15).

Conflict of Interest

The authors declare no competing interests.

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