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Effect of Propolis Applied to Goat Kids at Weaning Period on Heat Shock Protein Genes

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Abstract: In recent years, studies on the use of natural and organic additives have gained importance in goat breeding in order to prevent offspring losses and to encourage their growth and development by limiting the use of antibiotics. Especially the weaning period is a stressful period for kids and negative effects such as weight loss, increased susceptibility to viral and bacterial infections may be observed as a result of decreased nutrient intake and utilisation during this period. Considering these disadvantages that occur during the weaning period, it was thought that propolis would increase the potential to protect the health and welfare of kids during the weaning period due to its immunomodulatory, antioxidant, and anti-inflammatory effects. Therefore, we aimed to examine the expression levels of HSP27, HSP60, and HSP70 molecular chaperones that modulate the cellular stress response, which partially express the effects of propolis on weaning stress. Saanen kids were divided into propolis treated (n=10) and control (no propolis treatment; n=10) groups. The propolis-treated group received 0.4 cc propolis once a day for two weeks after weaning. Expression levels were calculated by $2^{-\Delta\Delta Ct}$ using the Pfaffl method and statistical significance levels were determined by Student t test. Blood samples were taken on the day of weaning and the following day to determine the effect of weaning stress on HSP27, HSP60, and HSP70 expression levels. The effect of propolis on weaning stress was examined in samples taken after two weeks of propolis treatment. The expression levels of HSP27 and HSP60 increased by approximately 2-fold during weaning stress, while HSP70 increased by 3.35-fold. When 0.4 cc propolis was applied to kids under weaning stress, a statistically significant downregulation of HSP27 level 1.08-fold, HSP60 level 1.56-fold, and HSP70 level 2.12-fold was obtained at the end of 2 weeks compared to the control group. Our study showed that propolis treatment decreased stress protein levels during weaning stress.

Keywords: *Goat kid, Heat shock proteins, Propolis, Weaning stress.*

Sütten Kesim Dönemindeki Oğlaklara Uygulanan Propolisin Isı Şok Protein Genlerine Etkisi

Özet: Son yıllarda keçi yetiştiriciliğinde, yavru kayıplarını önlemek ve antibiyotik kullanımını sınırlandırarak büyüme ve gelişmelerini teşvik etmek amacıyla doğal ve organik katkı maddeleri kullanımına yönelik çalışmalar önem kazanmıştır. Özellikle sütten kesme dönemi oğlaklar için stresli bir dönem olup, bu süreçte besin alımının ve yararlanımının azalması sonucu kilo kaybı, viral ve bakteriyel enfeksiyonlara karşı artan duyarlılık gibi olumsuz etkiler gözlemlenebilir. Sütten kesim döneminde ortaya çıkan bu dezavantajlar düşünüldüğünde propolisin immünmodülatör, antioksidan ve antiinflamatuvar etkilerinden dolayı sütten kesim döneminde oğlakların sağlığını koruma ve refahını arttıracağı düşünülmüştür. Bu nedenle çalışmada propolisin sütten kesme stresi üzerindeki etkilerini kısmen ifade eden, hücresel stres tepkisini modüle eden HSP27, HSP60 ve HSP70 moleküler şaperonların ekspresyon seviyelerini incelemeyi amaçladık. Saanen oğlakları propolis uygulanan (n=10) ve kontrol (propolis uygulanmayan; n=10) gruplarına ayrıldı. Propolis uygulanan gruba sütten kesildikten sonra 2 hafta boyunca günde bir kez 0,4 cc propolis verildi. Ekspresyon seviyeleri Pfaffl yöntemi kullanılarak $2^{-\Delta\Delta Ct}$ ile hesaplandı ve student t testi ile istatistiksel önem düzeyleri belirlendi. Sütten kesme stresinin HSP27, HSP60 ve HSP70 ekspresyon seviyeleri üzerindeki etkisini belirlemek için sütten kesim günü ve ertesi gün kan örnekleri alındı. Propolisin sütten kesme stresi üzerindeki etkisi, 2 haftalık propolis uygulamasından sonra alınan örneklerde incelendi. Oğlaklarda sütten kesme stresi sırasında HSP27 ve HSP60'in ekspresyon seviyeleri yaklaşık 2 kat artarken, HSP70 3,35 kat artmıştır. Sütten kesme stresi altındaki oğlaklara 0,4 cc propolis uygulandığında 2 haftanın sonunda kontrol grubuna göre HSP27 seviyesinde 1,08 kat, HSP60 seviyesinde 1,56 kat, HSP70 seviyesinde ise 2,12 kat istatistiksel olarak anlamlı downregülasyon elde edildi. Çalışmamız propolis uygulamasının sütten kesme stresi sırasında stres protein düzeylerini azalttığını gösterdi.

Anahtar Kelimeler: *Isı şok proteinler, Oğlak, Propolis, Sütten kesim stresi.*

Introduction

The economic success of goat breeding enterprises depends on reducing production costs and minimizing kid losses during birth and the neonatal period. Additionally, increasing the number of kids that reach a healthy and marketable age after weaning is also crucial. In dairy goat breeding, early weaning of goat kids is preferred to increase milk yield, reduce laborand feed costs, and promote functional reticulon-rumen development. However, this process is an imperative and a potential welfare concern. Weaning is a very stressful time involving adaptation to a new diet (Chauhan et al., 2019; Datt et al., 2023).

Stress leads to a decrease in feed utilisation or a negative effect on the efficiency of the gastrointestinal mucosa. By affecting growth rate, several other health problems may occur, such as developmental retardation, reduced weight gain, suppression of the immune system, or reduced function (Durosaro et al., 2023; Khan et al., 2016).

In response to stress, the unfolded protein response of the endoplasmic reticulum and mitochondria, cytosolic heat shock, hypoxic stress, and oxidative stress response occur. Following the response, defense pathways are activated, which initiate the activation of effector mechanisms that protect the animal from stress and repair the damage caused by stress. In this process, there is a decrease in protein translation levels and an increase in protein folding (Durosaro et al., 2023; Sala et al., 2017). Molecular chaperones are a family of proteins that facilitate and regulate the correct folding of proteins (Mogk et al., 2002). These molecular chaperones are present at normal levels in all eukaryotic and prokaryotic cells while maintaining normal biological activities. However, under many stressful conditions, chaperones are required to correctly fold proteins in the stress response and increase chaperone levels. These proteins are also called "stress proteins" because they increase their activities by protecting against the stress factor (Liberek et al., 2008; Öztürk et al., 2009).

HSP70 is particularly important in modulating and signalling the stress response within the HSP protein family (Korte et al., 2007). HSP70 increases cell tolerance to stressors, resists apoptosis, and reduces cell peroxidation and inflammatory damage (Ludwig et al., 1999). HSP60 is another important molecular chaperone that is encoded in the nucleus but expressed in the mitochondria (Grundtman et al., 2011). It has protective effects in many cells, exerting similar effects to HSP70 through different mechanisms (Otaka et al., 2006). Depending on changes in environmental conditions and stress factors, HSP60 synthesis increases, and HSP60 is transported into the cytosol and then appears on the cell surface where it acts as a "danger signal" for innate and acquired immunity (Choi et al., 2008; Grundtman et al., 2011). Recent studies have reported an association between extracellular HSP60 and the tissues immune responses and that it is upregulated in the inflammatory response (Grundtman et al., 2011; Liyanagamage and Martinus, 2020). HSP27 enhances antioxidant defenses by neutralizing the toxic effects of oxidized proteins in the cell and reducing the number of free radicals (Rogalla et al., 1999). Therefore, HSP27 levels increase when cells are exposed to oxidative stress (Mehlen et al., 1995). As a result, HSPs are one factor that maintains the balance between survival and an effective immune system in the organism during stress (Dangi et al., 2014). Many natural products such as purple garlic powder, thyme essential oil (Serrano-Jara et al., 2023), piperine (Satitsri et al., 2023) and grape seed meal by-product (Pistol et al., 2023) have been added to livestock diets to support immunity or prevent potential health problems after weaning. In goats, inoculation with rumen fluid (Belanche et al., 2020), palm oil wastewater (Nugroho et al., 2023), and probiotic (Chen et al., 2020) supplementation have been investigated as potential treatment options for immune response, growth performance, oxidative stress, hematological parameters, intestinal health and diarrhea after weaning. In recent years, the use of propolis in the livestock industry has become popular with the restriction of the use of antibiotics and synthetic drugs in this sector. Propolis supplementation was reported to improve growth rate and nutrient digestibility, reduced oxidative stress, and improved antioxidant capacity and immune response under stress conditions (Badawy, 2021; Sarker and Yang, 2010; Shedeed et al., 2019). The use of propolis as a natural alternative to ionophores in ruminants has been proposed by Stradiotti et al. (2004) and Oliveira et al. (2006). The effects of propolis supplementation on antiparasitic (Morsy et al., 2013), antibacterial (Ismael et al., 2019), feed efficiency (Zawadzki et al., 2011), milk yield and quality (Aguiar et al., 2014), in ruminants, have been studied with positive results. Given the positive effects of propolis under stress conditions, it is likely that a similar situation would occur under weaning stress.

The aim of our study was to determine the effect of propolis on changes in the stress system during the weaning period by comparing the gene expression levels of HSP27, HSP60, and HSP70 during the weaning period of goat kids given and not given propolis extract (in ethyl alcohol).

Material and Methods

The experiments were conducted according to the ethical guidelines for laboratory animal research and were approved by the Ethical Committee of Aydın Adnan Menderes University (64583101/2023/10).

Animal Material: The animal material of the study consisted of 20 Saanen goat kids born at the end of March 2023 from goat mothers synchronised in 2022 on a farm in İmamköy Efeler/Aydın. Goat kids rearing feed was used as concentrate feed, hay and alfalfa hay were used as roughage in the ration of weaned kids that weaning on July 2023 (approximately eight weeks old). The Saanen goat kids were divided into two groups: a control group (non-propolis-treated, n=10) and a propolis-treated group (n=10) with an equal ratio of males to females. The goat kids were homogeneously distributed among the groups according to their body weights (control, 10.40 kg ± 1.40; propolis-treated

group, 10.50 kg ± 1.72) and body condition scores (control, 1.84 ± 0.15; propolis-treated group 1.82 ± 0.14).

Study design: In our study, the complete weaning of the goat kids at eight weeks of age was planned by evaluating the literature data (Teh et al., 1984). The propolis group was given 0.4 cc of propolis by syringe once a day for two weeks as a dietary supplement (Manav and Yılmaz, 2023). Propolis extract in ethanol (The contents is given in table 1) was obtained from Idapolis Company (Turkey) within Çanakkale 18 Mart University Technopark.

Table 1. Contents and main constituents of the extract of propolis in ethanol.

Content and main components	Quantity (ppm)
Cumaric acid	19.50
Hydroxybenzoic acid	611.69
Caffeic acid	33.28
Catechin	0.17
Chlorogenic acid	0.35
D-(+) malic acid	71.87
Ellagic acid	18.90
Ferrulic acid	10.54
Gallic acid	2.46
Gentisic acid	0.53
Isorhamnetin	0.74
Isorhamnetin 3-O-glucoside	3.71
Isorhamnetin 3-rutinoside	12.35
Kaempferol	3.94
Myricitrin (Myricetin 3-O-rhamnoside)	1.35
p-hydroxy benzoic acid (4 hydroxy benzoic acid)	278.05
p-cumaric acid	15.36
Protocatechuic acid	4.58
Quercetin (Quercetin 3-O-rhamnoside)	3.63
Quercetin 3-D-xyloside	0.69
Quercetin 3-O-rutinoside hydrate	2.50
Suscinic acid-butanedionic acid	5.97

Goats have a low feed efficiency, a low level of immunity and a high risk of diarrhoea during the weaning period. There are different doses for propolis application in the literature studies, but in our study the 0.4 cc application was preferred due to its effect on both immunity and antidiarrhoeal effect (Manav and Yılmaz, 2023; Sadek et al., 2020).

Collection blood sample: Blood samples were taken from the animals' jugular veins (*vena jugularis*) into EDTA tubes. To determine the effect of weaning stress on HSP levels, goat kids were weaned at 8 weeks of age by blood sampling. Then goat kids were separated from their mothers and blood samples were taken again 24 hours later. After two weeks of propolis treatment, blood was collected from both the propolis-treated group and the control groups. As soon as the samples were collected, they were transported to the laboratory attention to the cold chain.

RNA isolation from blood and cDNA synthesis: Total RNA was isolated from whole blood using a commercial RNA isolation kit (RiboEX, GeneAll, Korea) according to the protocol, and the RNA was obtained RNAs and stored at -80ºC until cDNA synthesis. The concentration of RNA samples was determined at µg/µl level by measuring at 260 nm wavelength in a microplate reader (Multiskan™ FC Microplate Photometer, Thermo Fisher, Finland) and diluted to 1000 ng with DNase/RNase-free water.

RNA samples were converted to cDNA using a cDNA synthesis kit (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems, USA) according to the kit procedure in an Applied Biosystems 2720 Termalcycler (Singapore).

Primer sequences: To determine the effects of propolis applied to goats on heat shock proteins during the weaning process at the gene level, gene expression levels were determined by using primers goat-specific HSP27, HSP60, HSP70, and β-actin genes from the generated cDNAs. Primers for the reference gene β-actin and the reverse primer for the HSP27 gene were designed using the Primer 3 program. The primer sequences for the HSP60 and HSP70 genes were taken from Dangi et al. (2014), and for forward primer HSP27 genes from Tsugami et al. (2023) (Table 2).

Table 2. Primer sequence

Target Gene	Accession number	Primer sequences (5'→3')			Amplicon size
HSP27	XM_018040903.1	F	5'-TCACTCGCAAATACACGCTG-3'	20 bp	142 bp
		R	5'- AAGGTGACGGGAATGGTGAT-3'	20 bp	
HSP60	XM_018061271.1	F	5'-ACTGGCTCCTCATCTCACTC-3'	20 bp	147 bp
		R	5'-TGTTCAATAATCACTGTCCTTCC-3'	23 bp	
HSP70	NM_001285703.1	F	5'-GACGACGGCATCTTCAAG -3'	18 bp	132 bp
		R	5'-GTTCTGGCTGATGTCCTTC-3'	19 bp	
β-actin	NM_001314342.1	F	5'-AGTTCGCCATGGATGATGA-3'	19 bp	54 bp
		R	5'-TGCCGGAGCCGTTGT-3'	15 bp	

Determination of relative gene expression levels: The expression levels of HSP27, HSP60, and HSP70 genes were determined by the qRT-PCR method using the A.B.T.™ 2X qPCR SYBR-Green MasterMix (Turkey) commercial kit in LigthCycler®480 device (Roche, Germany). Each cDNA sample was tested three times, and the cycle threshold (Ct) was determined. The RT-PCR condition was an initial incubation at 95 ºC for 5 minutes, followed by 40 cycles at 95 ºC (15 s) and 60 ºC (30 s). The Ct value obtained for each sample was normalised to β-actin as a reference gene. The results for the target genes in calculating the relative quantification of gene expression were expressed by the formula $2^{-\Delta\Delta Ct}$ using the Pfaffl method (Livak and Schmittgen, 2001).

Statistical Analyses: All statistical analyses and calculations were performed using MS-Excel 2019 and SPSS for Windows Ver. 25.0 (SPSS Inc., Chicago, IL., USA). In statistical decisions, a level of $p<0.05$ was accepted as an indicator of significant difference. Descriptive analyses were performed as mean and standard deviation for all parameters. Continuous variables were assessed for normality using the Shapiro-Wilks test. Comparisons between groups were made using the Student T test for normally distributed data.

Results

The weekly body weight and body condition scores of the goat kids at weaning time and during propolis treatment are summarised in the table (Table 3).

Table 3. Body weight and body condition scores of the goat kids.

		Initial n:10	1. week n: 10	2. week n: 10	*p*
Control group	LW	10.40±1.40[a]	11.43±1.50[b]	11.70±1.60[b]	*
	BCS	1.82±0.14	1.70±0.17	1.86±0.18	IN
Propolis-treated group	LW	10.50±1.72[a]	11.60±1.64[ab]	12.25±1.75[b]	*
	BCS	1.84±0.15	1.75±0.14	1.85±0.16	IN

a, b: Differences between averages with different letters on the same line were statistically (mean ± SE) significant (*p<0.05), IN; insignificant. LW; live weight BCS; body condition score

Effect of weaning stress before propolis application on gene levels: The relative change in HSP27, HSP60 and HSP70 genes due to weaning stress was determined in the 8th week weaning samples. These were taken before weaning and the next day after the kids were weaned. At this stage, propolis application was not started and 20 Saanen kids were included in the calculation in order to increase the number of animals. The levels of expression were determined by the ratio of the data obtained for each HSP protein as a result of the qPCR to that of β-actin. The relative expression levels of each gene were calculated using the Pfaffl method ($2^{-\Delta\Delta Ct}$), with the expression levels of the pre-weaning samples assumed to be 1 (Table 4).

Table 4. Expression levels of HSP proteins in samples taken before and after weaning ($2^{\Delta Ct}$), (ΔCt= Ct (HSP gene) – Ct (β-actin)).

	HSP proteins expression levels			HSP proteins relative expression		
	HSP27	**HSP60**	**HSP70**	**HSP27**	**HSP60**	**HSP70**
Pre-weaning	1,32E-03	2,13E-03	5,63E-02	1	1	1
Post-weaning	2,35E-03	4,95E-03	1,89E-01	1.77	2.32	3.35

The relative expression level of HSP70 protein was detected 3.35-fold up-regulated after weaning 24 h compared to before weaning. In the same sample, the expression levels of HSP27 and HSP60 significantly increased in the 1.77 and 2.32-fold up-regulation, respectively (Figure 1).

To determine the effect of propolis on the weaning stress end of the application, the changes of HSP27, HSP60 and HSP70 genes were examined after weaning compared to the control group. Relative gene expression levels in the samples taken were calculated with Pfaffl method ($2^{-\Delta\Delta Ct}$) accept to expression levels of the control group without propolis application as 1. According to our results, a 1.08-fold significant down-regulation was observed at the HSP27 expression level. At HSP60 and HSP70 expression levels, a 1.56and 2.13 fold downregulation was observed with propolis application, respectively (Figure 2).

In addition, it is thought that changes in the expression levels of HSP proteins may occur during the weaning process of the control group. Therefore, HSP protein levels were compared in samples taken from the control group after weaning and in samples taken two weeks later. The relative gene expression level of HSP70 showed a 1.03-fold change, while the relative expression levels of HSP27 and HSP60 genes were 1.07 and 1.08, respectively. When the samples from the group allocated for propolis treatment after weaning and at the end of propolis treatment were compared, 2.20-fold down-regulation was detected in HSP70 level and 1.16 and 1.69 changes in HSP27 and HSP60 genes, respectively.

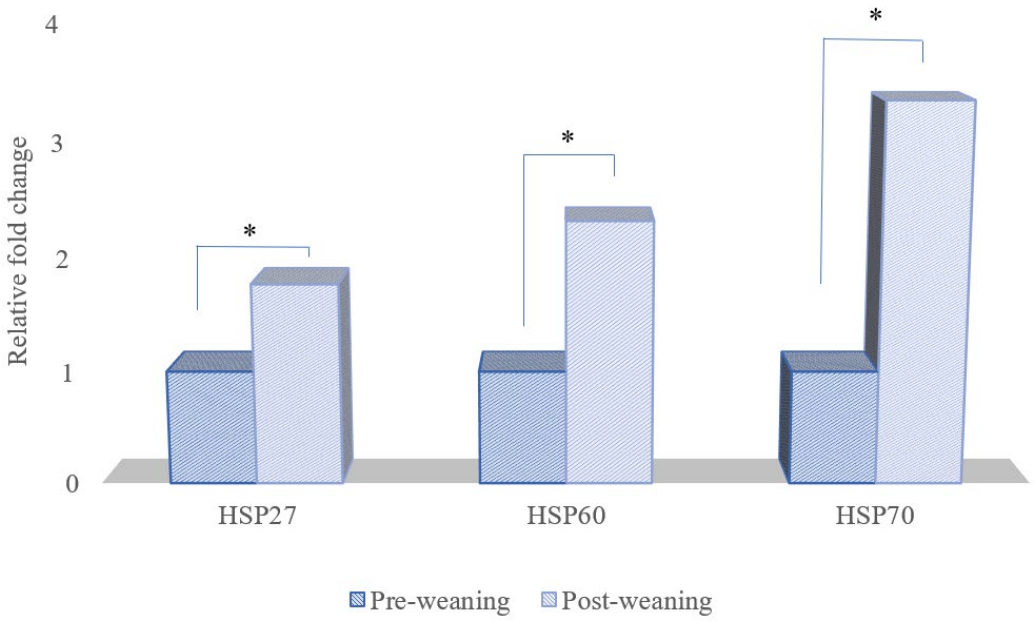

Figure 1. Effect weaning stres on HSP27, HSP60 and HSP70 genes levels (*p <0.001; n=20).

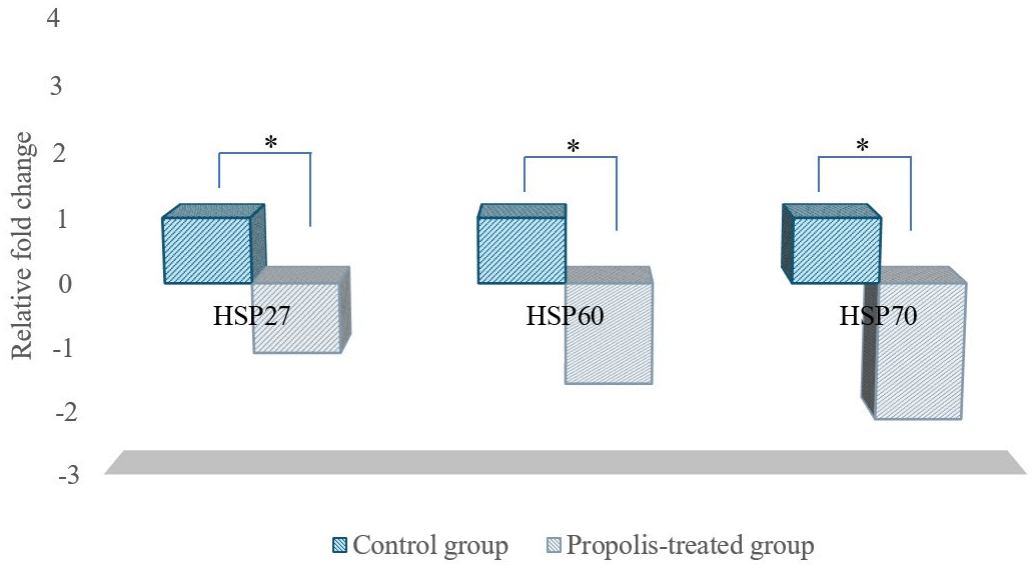

Figure 2. The effect of propolis supplementation on HSP27, HSP60, HSP70 levels in goat kids under weaning stress (*p <0.001; n=10).

Discussion and Conclusion

In goat husbandry, many factors in the breeding process affect animal health and create conditions that can cause stress. It is complicated for animals to adapt to changes in feed intake, diet form and composition, nutrient digestibility, and social and physical environment during the weaning process (Degroote et al., 2020). During the weaning process, the care needs of the kid increase. Weaning is another critical stage in ruminant production, where losses increase after the neonatal period (Baldwin et al., 2004; Khan et al., 2016). Therefore, some measures taken between these periods will prevent offspring losses and contribute significantly to the offspring's quality of life and welfare (Datt et al., 2023). In recent years, studies to minimize the disadvantages of weaning practices in different species have attracted great interest (Cox and Cooper, 2001; Jeppesen et al., 2000). Many studies have addressed problems such as weight loss, decreased feed conversion, and susceptibility to viral and bacterial infections during weaning (Baldwin et al., 2004; Belanche et al., 2020; Chen et al., 2020). However, studies determining the changes that may occur in heat shock proteins due to weaning stress are limited.

Under normal conditions, HSP acts as a chaperone molecule, is increased expressed under stress conditions, and plays a role in maintaining cell homeostasis (Kresnoadi et al., 2020). It is known that HSP expressions are affected by endogenous physiological factors (Ehrenfried et al., 1995) and environmental factors (Yılmaz et al., 2018). In addition, changes in HSP expression have been determined in situations such as exercise, weaning, transport, high temperature, and exposure to toxins (Hussain et al., 2021). It has been reported that HSP expressions change in the gastro-intestinal system with weaning and expression may be affected depending on the stage after weaning and changes in the gastrointestinal system (Arvans et al., 2005). Apart from the gastrointestinal tract, changes in HSP expression levels have been detected in various tissues, such as the myocardium, kidney, and longissimus dorsi muscle (Li et al., 2018). There is no literature on the changes in HSP proteins in circulation during weaning in ruminants. Therefore, in our study, we planned to take samples before and 24 hours after weaning to determine the effect of weaning stress on the expression of HSP proteins. When the studies conducted in ruminates were examined, Although the intensity of weaning stress varies between species, it has been reported that both behavioral and physiological stress is more pronounced in the first 24-48 hours following an abruptly weaned (Lynch et al., 2019; Kazemi et al., 2023; Vickery et al., 2023). Especially the first 24 hours were considered because cortisol levels affect the expression of HSPs (LeBlanc et al., 2012).

There was no difference between the groups in the goat kids whose weekly live weights were measured during the study period. When the literature information was evaluated, Abd-Allah and Daghash (2019) reported that the weaning weights of calves fed with 50 mg propolis / head / day in addition to the ration were 7.7 kg more than the control group. Cécere et al. (2021) added 150 µl/day propolis to the milk of lambs for 42 days and reported a difference of approximately 3 kg in live weight. The reason for the increase in live weight in our study is that our application period may have been short in comparison to other studies.

In our results, an increase in the level of HSP27, HSP60, and HSP70 was observed. Li et al. (2018) investigated the effect of weaning age on HSP proteins in piglets and determined HSP27 level was unaffected by weaning stress, while the HSP70 level increased. High phenolic content additives inhibit HSP27 and HSP70 (Roussou et al., 2004). Since propolis has a high phenolic content, it is thought that HSP27 and HSP70 may determine its effects during the weaning process. However, Cécere et al. (2021) and Shedeed et al. (2019) proved that propolis application reduced serum ROS levels and increased antioxidant levels according to a study in sheep. It is thought that propolis plays a role in balancing the oxidative stress caused by stress in this period and causes a positive effect on stress proteins.

Weaning stress causes an increase in inflammatory molecules and affects intestinal health. Gut microbiota changes, intestinal dysfunction occurs, antioxidant system is inhibited (Hussain et al., 2021). Studies report that polyphenols positively affect the changes at the molecular level mediated by the intestinal microbiota under the influence of weaning stress (Moreno-Indias et al., 2016; Mosele et al., 2015; Selma et al., 2009;). Many studies have documented successful feeding strategies with weaning stress and polyphenol supplementation in pigs (Fiesel et al., 2014). Unfortunately, there is no such extensive literature on buffalo, cattle, goats and sheep. Growth and development parameters and immunoglobulins were evaluated as immune responses in calves treated with propolis during weaning (Sarker and Yang, 2010).

Our results show that propolis, which has been used in human health for centuries due to its strengthening the immune system, accelerating the healing and regeneration of tissues, antioxidant and anti-inflammatory effects, is suitable for animal husbandry. Many studies have investigated its use in treating diarrhoea, as an alternative to antibiotics, and its effects on growth and development parameters. The effect of propolis during the weaning process, which is one of the critical growth periods of goat kids, has not been investigated. In addition, the results of our study indicate the importance of weaning stress and possible dietary interventions with polyphenols to improve offspring growth and production in ruminants.

Although we do not know which molecular pathway the application of propolis, rich in polyphenols, affects the level of HSP proteins, we can say that it can minimize the effects of weaning stress with the decrease in HSP levels. Supplementation of feed with propolis is known to reduce HSP70 levels in animals. A similar effect was reported in our study to be effective in weaning stress. However, further studies are needed to define the effect of propolis application on the rumen microbiome, its interaction with different diets and its long-term effects on animal productivity during weaning.

Conclusion

During the weaning period, some disadvantages may occur on the immune system and general health status of kids. During this period, the immune system may weaken due to stress and nutritional changes, disease resistance may decrease and growth performance may be adversely affected. In this context, considering the immunomodulatory, antioxidant and anti-inflammatory effects of propolis, it can be recommended to be used to support the health of kids during the weaning period. Thanks to these effects of propolis, it can be aimed to alleviate the negative effects encountered during the weaning period and to improve the general health of animals. In accordance with the aforementioned data, the administration of propolis during the weaning period has been demonstrated to facilitate the healthy development of goats.

Limitations

In this study, circulating levels of HSPs could not be determined, only changes in gene levels were measured. In addition, the effects of weaning age were not examined to better understand the effect of propolis on weaning stress.

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Conflict of Interest

The authors stated that they did not have any real, potential or perceived conflict of interest.

Ethical Approval

This study was approved by the Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (19.01.2023, 64583101/2023/10 Number Ethics Committee Decision). In addition, the authors declared that Research and Publication Ethical rules were followed.

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